PhD. 36717


# HIV, body composition, bone and vitamin D status in South African women

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Dissertation submitted for the degree of Doctor of Philosophy

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Title: HIV, body composition, bone and vitamin D status in South African women.

This dissertation is submitted for the degree of Doctor of Philosophy.

Declaration: This dissertation is the result of my own work and includes nothing that is the outcome of work done in collaboration except where specifically indicated in the text. No part of the work has been submitted for any other qualification.

Matthew Hamill

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"I had forgotten how much light there is in the world, till you gave it back to me."

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## Abstract

Cross sectional and observational data suggest that HIV-positive individuals and those receiving antiretroviral (ARV) therapy are prone to higher rates of osteoporosis and osteopaenia than HIV-negative individuals. Likewise, HIV-positive individuals often have low vitamin D status. Evidence is emerging more generally of a strong association between HIV infection and poor bone health. There is also evidence that treatment with ARV therapy (ART) and suboptimal vitamin D status may exacerbate this problem (Brown *et al*, 2006a, 2010). But, to date, causal relationships have not been fully established. This thesis explores the interactions between these separate factors and provides novel data about the effects of HIV infection and its treatment, on bone health in a particular group of black, South African women.

Bone loss and poor vitamin D status in the context of HIV infection are important global health issues because these conditions may affect millions of individuals. If HIV-associated bone loss is causally associated with an increased risk of bone fracture then it is possible that there will be an epidemic of HIV-associated fractures in coming decades, particularly in the developing world, including Africa. Study data have so far often been limited by several factors, including cross-sectional design, absence of control groups, a preponderance of attention to bone outcomes in males and in Caucasians, and a lack of good quality data in Africans living in Africa.

This study aimed to assess the magnitude of HIV- and ART-associated changes in areal bone mineral density (aBMD), size-adjusted bone mineral content (SA-BMC) and vitamin D status in adult, premenopausal women living in Johannesburg, South Africa. Ninety-eight HIV-negative (Negative reference: Nref) and 149 HIV-positive women were enrolled to allow for comparison between groups. The HIV-positive women were recruited into those eligible to start ART (Positive low $CD_4$: Plow, n=75) and those unlikely to require ART (Positive preserved $CD_4$: Ppres, n=74) during a 12-month follow-up period. The design was longitudinal with visits at 0, 6 and 12 months for measurement of body composition, bone measures and dietary assessment. Blood and

urine samples were collected for the evaluation of relevant musculoskeletal analytes, including 25(OH)D at each time point. Most women (>80%) who received ART during the course of the study received South African standard first-line therapy consisting of lamivudine, tenofovir and efavirenz.

A *post hoc* analysis of possible effects of ART was performed by retrospectively dividing HIV-positive women into ART-unexposed (n=66) and ART-exposed (n=74).

At baseline there was a high prevalence of overweight with 65%, 65% and 44% with BMI >25 kg/m$^2$ in Nref, Ppres and Plow respectively. Plow had lower weight, BMI, fat mass, lean mass, waist and hip circumferences than the other groups. Nref and Ppres were not different from each other. There were no differences in aBMD or SA-BMC between groups at baseline and no significant differences in vitamin D status between the groups. The mean ±SD serum 25(OH)D concentrations were 59.7 ±16.5, 59.2 ±16.5 and 61.6 ±22.3 nmol/l in Nref, Ppres and Plow respectively. Plow had significantly lower serum albumin concentration ($p<0.0001$) and higher serum phosphate concentration ($p<0.0001$). The magnitude of differences in serum phosphate was: Ppres-Nref = 12.7 ±2.9%; Plow-Nref = 20.3 ±2.9% and Plow-Ppres = 7.6 ±3.1% ($p<0.001$).

Tubular maximum Reabsorption of Phosphate/Glomerular Filtration Rate (TmP/GFR) was 11.2 ±3.2% and 27.4 ±3.2% respectively greater in Ppres and Plow than Nref ($p<0.0001$), and higher in the Plow compared to Ppres 16.2 ±3.4%, ($p=0.0002$). Serum alkaline phosphatase and urine phosphate to creatinine ratio were not significantly different ($p>0.05$).

At the 12-month follow-up, Plow subjects remained lighter than their Nref and Ppres counterparts. However, there was a 3.9 ±0.9% increase in mean weight in the Plow group over 12 months ($p<0.001$), which represented 10.2 ±0.8% ($p<0.001$) increase in fat, rather than lean, mass accumulation. There were significant mean decreases in aBMD and SA-BMC in Plow subjects, and those exposed to ART of the order of 2-3% at total hip, femoral neck and lumbar spine.

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There were no significant differences in mean vitamin D status between the groups and no significant changes, the mean 25(OH)D concentrations were 63.3 ±17.7, 66.0 ±18.4 and 61.1 ±20.1 nmol/l in Nref, Ppres and Plow respectively. Serum albumin concentrations had risen by a mean of 9.1 ±1.1% in the Plow group to reach comparable concentrations with the other groups. Alkaline phosphatase activity had significantly risen in the Plow group compared with the other groups ($p<0.001$). Serum phosphate concentration remained higher in Plow than the other groups, though the mean value had not increased. Serum phosphate had significantly increased in Nref from baseline to 12 months 7.0 ±2.3% ($p=0.05$) and non-significantly in Ppres 5.2 ±2.4%. TmP/GFR had declined from baseline by 11.2 ±3.6% in Plow and non-significantly increased in Nref and Ppres (6.4 ±3.3% and 3.8 ±3.5% respectively).

These data suggest that HIV infection in South African women is associated with differences in body composition but not with differences in bone measures or vitamin D status. However, being in the Plow group, and ART exposure, was associated with a significant decrease in mean aBMD and SA-BMC, of the order of 2-3%, over 12 months of observation at the hip, femoral neck and lumbar spine. These decreases, in young women, exceed those seen in early menopause, which is of the order of 1-2% annual decrease. The decreases were evident despite the fact that HIV-positive women exposed to ART had increases in fat mass, weight and serum albumin and alkaline phosphatase over time. In this group serum phosphate concentration and TmP/GFR decreased after the introduction of ART, suggesting an effect of ART on renal phosphate handling. ART exposure was not associated with change in vitamin D status.

In the *post hoc* analysis the biochemical results in ART-unexposed compared to ART-exposed was very similar to that in Ppres compared with Plow.

Further studies to assess skeletal effects over a longer time in HIV-positive, ART-exposed and naïve women are warranted. Studies are also required in post-menopausal women, children and men. Given the high prevalence of overweight and obesity recorded

in the study population, there may also be a need for interventions to reduce cardio-metabolic disease risk in this population.





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## Abbreviations

**$1,25(OH)_2D$**: 1,25-dihydroxyvitamin D
**3TC**: Lamivudine
**25(OH)D**: 25-hydroxyvitamin D
**ABC**: Abacavir
**aBMD**: Areal bone mineral density
**AIDS**: Acquired Immunodeficiency Deficiency Syndrome
**ALP**: Alkaline phosphatase
**ART**: Antiretroviral therapy
**ARV**: Antiretroviral
**ATZ/r**: Atazanavir/ritonavir
**AZT**: Zidovudine
**BA**: Bone area
**BALP**: Bone alkaline phosphatase
**BMC**: Bone mineral content
**BMD**: Bone mineral density
**BMI**: Body mass index
**BSP**: Bone sialoprotein
**Bt20**: Birth to Twenty (Cohort, Johannesburg)
**BTM**: Bone turnover markers
**$CD_4$**: Cluster of differentiation 4
**CDC**: Centers for Disease Control and Prevention
**CTx**: Serum collagen type 1 cross-linked C-telopeptide
**CYP**: Cytochrome P450
**D4T**: Stavudine
**DBP**: D binding protein
**DKK-1**: Dickkopf-1
**DIPART**: Vitamin D individual patient analysis of randomized trials
**DMP1**: Dentin matrix protein 1
**DNA**: Deoxyribonucleic acid
**DPHRU**: Developmental pathways for health research unit
**DXA**: Dual energy X-ray absorptiometry
**EFV**: Efavirenz
**FFQ**: Food frequency questionnaire
**FGF-23**: Fibroblast growth factor-23
**FN**: Femoral neck
**FPM**: Food photographic manual
**FSH**: Follicle stimulating hormone
**FTC**: Emtricitabine
**GFR**: Glomerular filtration rate
**Gp**: Group
**Gph**: Group hierarchical
**GRID**: Gay related immune deficiency
**HAART**: Highly active antiretroviral therapy
**HIV**: Human Immunodeficiency Virus
**HNR**: Human Nutrition Research
**ID**: Identification
**IDh**: Identification hierarchical
**IL**: Interleukin
**IRMER**: Ionising radiation (medical exposure) regulations
**ITT**: Intention to treat
**LS**: Lumbar spine
**MRC**: Medical Research Council
**MTCT**: Mother-to-child transmission
**NFkB**: Nuclear factor kappa beta
**NHANES**: National health and nutrition examination survey

**NNRTI**: Non-nucleoside reverse transcriptase inhibitor
**NRTI**: Nucleoside reverse transcriptase inhibitor
**NTx**: N-terminal telopeptide of type I collagen
**NVP**: Nevirapine
**OPG**: Osteoprotegerin
**PCP**: Pneumocystis pneumonia
**PHEX**: Phosphate regulating neutral endopeptidase
**PHRU**: Perinatal HIV research unit
**PI**: Protease inhibitors
**PMTCT**: Prevention of mother-to-child transmission
**POCT**: Point of care test
**pQCT**: Peripheral quantitative computed tomography
**PrEP**: Pre-exposure prophylaxis
**QA**: Quality assurance
**QUS**: Quantitative ultrasound
**RANKL**: Receptor activator of nuclear factor-kappa B ligand
**RECORD**: Randomised evaluation of calcium or vitamin D
**RNA**: Ribonucleic acid
**RoI**: Region of interest
**RR**: Relative risk
**RT**: Reverse transcriptase
**SA-BMC**: Size adjusted bone mineral content
**SA MRC**: South African Medical Research Council
**SD**: Standard deviation
**SE**: Standard error
**SIV**: Simian immunodeficiency
**SMART**: Strategies for management of anti-retroviral therapy
**SSA**: Sub Saharan Africa
**SUN**: Study to understand the natural history of HIV and AIDS in the era of effective therapy
**TB**: Tuberculosis
**TDF**: Tenofovir
**TH**: Total hip
**TmP**: Tubular maximum reabsorption rate of phosphate
**TmP/GFR**: Tubular maximum reabsorption rate of phosphate to glomerular filtration rate
**TNFα**: Tumour necrosis factor alpha
**TRAcP**: Tartrate-resistant acid phosphatase
**UNAIDS**: Joint United Nations programme on HIV/AIDS
**UVB**: Ultraviolet B
**VCT**: Voluntary counselling and testing
**VDR**: Vitamin D receptor
**WB**: Whole body
**WBLH**: Whole body less head
**WBS**: Women's bone study
**WHI**: Women's health initiative
**WnT**: Wingless-type MMTV integration site family, member 1

# 1 Background to the PhD

## 1.1 Background information on HIV infection, osteoporosis, and vitamin D status

HIV infection, osteoporosis, and poor vitamin D status are all enormous global health problems, affecting millions of people and requiring careful study because of the ill health that results when left untreated. All these conditions are associated with increases in individual morbidity and mortality and decreases in quality of life, and huge financial healthcare and societal costs.

Historically, the mortality and morbidity associated with HIV infection have been due to opportunistic infections, such as tuberculosis (TB). However, the availability of drugs used for antiretroviral therapy (ART), in the management of HIV infection, has seen the life expectancy of those infected increase dramatically (Palella *et al*, 1998). Consequently, the focus of healthcare for these patients has shifted from infectious disease to non-communicable disease as a cause of morbidity and premature mortality. This has been accompanied by an increased recognition of HIV-associated osteoporosis as distinct from the more 'typical' HIV-associated bone pathologies such as avascular necrosis of the hip (Mehta *et al*, 2013). There is a growing clinical perception that bone loss is occurring at an earlier age in HIV-positive than in HIV-negative adults, and that HIV is associated with premature ageing.

HIV is a global pandemic; however, the burden of HIV/AIDS lies squarely among women in Sub-Saharan Africa, notably in South Africa, which has a very large total burden of disease. As elsewhere in the world, the demography of HIV infection in South Africa is rapidly changing with the emergence of an ageing cohort of infected individuals. Consequently, the country faces a 'double burden' of communicable and non-communicable diseases in the adult population.

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## 1.2 Extent of the problem

### 1.2.1 Osteoporosis

Osteoporosis is a systemic skeletal disorder characterised by "low bone mass, microarchitectural deterioration of bone tissue leading to enhanced bone fragility, and a consequent increase in fracture risk" (Consensus Development Conference, 1991). It is usually diagnosed using imaging techniques, predominantly Dual Energy X-ray absorptiometry (DXA). The World Health Organization (WHO) has suggested a working definition of osteoporosis and osteopaenia based on a comparison of an individual's areal bone mineral density (aBMD) and the mean (SD) aBMD at the time of peak bone mass in 30-year-olds. Osteoporosis diagnosis uses a T-score (defined as (x-mean)/SD) of less than -2.5 SD and osteopaenia as a T-score of between -1.0 and -2.5 SD relative to that expected at the time of peak bone mass. In postmenopausal women, the odds ratio for lifetime risk of hip fracture is 2.6 for every SD decrease in aBMD (Marshall *et al*, 1996).

Osteoporosis is the most common metabolic bone disease in the world. An estimated 10 million of the US population were affected in 2005 and more than 14 million are predicted to be affected by 2020 (Burge *et al*, 2007). In 2010, it was estimated that 1.8 million were living with osteoporosis in the UK and this number is predicted to rise to 2.1 million by 2020 (Gauthier *et al*, 2011). The prevalence of osteoporosis is greatest in women, and increases with age. In addition, as the numbers of older people in a population increase, so too does the prevalence of osteoporosis. The burden of osteoporotic fragility fractures is compounded by the fact that osteoporosis is frequently under-diagnosed and is left under- or un-treated (Walker-Bone, 2012).

The global burden of osteoporosis presents a pressing public health problem, particularly in the elderly. Aside from significant associated morbidity and mortality, the estimated annual costs to health services are, for example, £1.8 billion in the UK and €30 billion in Europe (Pollock *et al*, 2009). National (Lippuner *et al*, 2005) and global costs of fracture are enormous, estimated in 1990 at US$34.8 billion, and expected to increase substantially by the middle of this century (Harvey *et al*, 2010). The predicted increases

by 2050 are greatest in Africa, Asia, and Latin America (Harvey *et al*, 2010). More up-to-date estimates of global disease burden are lacking. It is estimated that in Europe each year 179,000 men and 611,000 women will suffer a hip fracture. Data for Africa are very sparse, so to use a different developing region, Latin America, as an example, there will be an estimated 655,648 hip fractures in 2050, with an estimated direct cost of $13 billion (IOF, 2009). The International Osteoporosis Foundation estimates that between 1990 and 2000, there was an almost 25% increase in hip fractures and that by 2050, the worldwide incidence of hip fracture in men will increase by 310% and 240% in women (Gullberg *et al*, 1997; IOF, 2009).

In South Africa, the focus of the research described in this thesis, the prevalence of osteoporosis in white, Asian, and mixed-race populations "appears to be similar to that of developed countries, although no accurate fracture data exist. As in the USA, hip osteoporosis is less prevalent in … black populations, although vertebral bone mass, and possibly also fracture prevalence, in black and white South Africans appear to be similar" (Hough, 2010). The National Osteoporosis Foundation of South Africa recognises that there is a need for further research into racial differences in osteoporosis and fragility fracture risk. More recently, Chantler *et al* have described differences in aBMD between black and white South African women, with whites having lower hip but higher lumbar spine aBMD than blacks. These differences were attributed to differences in body composition, lifestyle, and socio-economic factors (Chantler *et al*, 2012).

Osteoporosis in postmenopausal women is considered to be a consequence of increased bone remodelling due to oestrogen deficiency, whereby bone resorption is uncoupled from, and outstrips, bone formation. In older women and men, vitamin D deficiency and hyperparathyroidism are important risk factors for low bone mass; other 'traditional' risk factors include smoking, excess ethanol, and low physical activity. Low lean mass, is also a risk factor for osteoporosis development (Rikkonen *et al*, 2012).





The pathophysiology of low bone mass, and increased fracture risk, in younger adults is less well defined but may include slower bone mass acquisition and lower peak bone mass than in healthy individuals, or be the result of a specific medical condition such as coeliac disease (Ferrari *et al*, 2012). The resultant low bone mass can result in an increased risk of fracture at an earlier age than usually associated with fragility fractures and/or increased risk later in life (in the 7$^{th}$ decade and beyond).

More detailed information regarding osteoporosis and its underlying bone biology is given in Chapter 2.

### 1.2.2 HIV infection

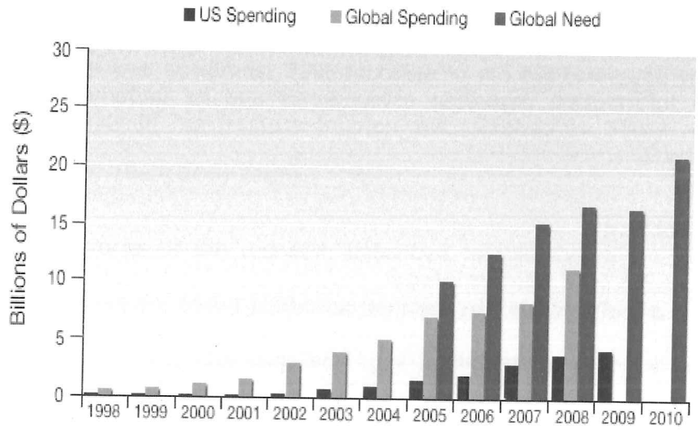
#### 1.2.2.1 HIV epidemiology

HIV infection is a multisystem disease that can affect every organ in the body, including those involved in bone metabolism, e.g. the gut, kidneys, and parathyroid glands (Hellman *et al*, 1994). In addition, HIV can have a deleterious effect on nutritional status and/or intake, body composition (Segatto *et al*, 2012), and the ability to be physically active, all of which have important, potentially negative consequences for bone health.

Worldwide, there were an estimated 33 million people living with HIV infection in 2007, with an estimated annual incidence of 2.7 million, and with Sub-Saharan Africa the most affected region, accounting for 67% of all infections and 72% of all HIV-related deaths (UNAIDS, 2008a). In 2011, UNAIDS estimated that there were 34.2 million people living with HIV (UNAIDS, 2011). Whilst the global epidemic has been stabilising since 2000, the prevalence is continuing to rise because of access to ARV therapy prolonging survival, combined with on-going new infections (UNAIDS, 2008a). Within Sub-Saharan Africa, the greatest burden of HIV infection is concentrated in the Southern Africa region where prevalence in adults ranges between 15 – 28% (UNAIDS, 2008a) and where, as a result, there has been a decline in life expectancy (Logie, 1999), although there is evidence that these declines may be slowing or reversing (Hontelez *et al*, 2012; Bor *et al*, 2013).

The estimated global annual expenditure for treatment and prevention of HIV is $13.7 billion (2008 data) (UNAIDS, 2008b; Global Health Council, 2010) (Figure 1-1) with predictions that by 2031 the costs will reach $35 billion (Hecht *et al*, 2009):

**Figure 1-1 Estimated US and global expenditure on HIV/AIDS**




Estimated US and global expenditure on HIV/AIDS compared with the global need for universal access to ART up to 2010 (Global, 2010)* (*Global Health Council, no longer operational so unable to apply for copyright permission)

#### 1.2.2.2 HIV and chronic diseases of old age

Recent estimates in the USA suggest that 50% of HIV-positive individuals will be aged over 50 years by 2015 (Yin *et al*, 2009). In the UK it is estimated that 8% of women and 19% of men with HIV infection were over 50 years of age in 2006 (Pollock *et al*, 2009). The expectation is that older individuals will develop conditions such as cardiovascular disease and osteoporosis (Goulet *et al*, 2007) in a similar manner to the HIV-negative ageing population, although these conditions may occur at a younger age. For example, proxy markers for increased cardiovascular disease (CVD) risk, such as D-dimer concentrations in blood, appear to be increased in HIV-infected patients (Aberg, 2012). This suggests that end organ disease (e.g. myocardial infarction) may have an increased prevalence in HIV-infected patients as this cohort continues to age (Aberg, 2012). Effros *et al* describe this as HIV infection "compressing the ageing process" (Effros *et al*, 2008). The full spectrum of age-related disease burden in HIV-positive patients has not yet been fully elucidated because the population of affected individuals is relatively young at





the present time when compared with ageing populations in general, although the ageing of these patients will inevitably make this relationship clearer in coming decades. 2012 data suggest that in Sub-Saharan Africa, if current coverage (i.e. provision of ART to those who are eligible) of ART continue to increase at the current rate, the ratio of HIV-positive patients aged over 50 years will increase from 1:7 of HIV-positive adults (2011) to greater than 1:4 (2040) (Hontelez *et al*, 2012).

A more detailed description of HIV infection and its underlying biology is given in Chapter 2.

#### 1.2.2.3 ARV therapy

Effective, combined ARV drugs have been in use widely in the West since the mid-1990s. ART usually consists of a combination of three drugs: two nucleoside reverse transcriptase inhibitors (NRTI) and one non-nucleoside reverse transcriptase inhibitor (NNRTI) or protease inhibitor (PI). Numerically, the vast majority of the population with access to ART are in the developing world, and most patients will access an NNRTI-based regime since this is significantly cheaper than a PI-based regime. In most European countries the first-line ART regime is also NNRTI-based. It was estimated that in 2010, 15 million individuals were accessing ART globally. There is, however, a huge variation in estimates of ART-coverage, with some countries managing only 1% (Madagascar) and others >95% (Comoros) in 2010 (UNAIDS, 2011). This compares with 66% coverage in South Africa as of 2011.

ART is largely able to reverse the deleterious effect of HIV infection by suppressing viral replication; this allows for restoration and near normalisation of immune function. HIV infection damages the immune system and is mediated by viral infection of $CD_4$-positive immune cells (see Section 2.1.1). However, there are multiple effects on body systems, particularly kidney, liver, and bone, and long-term ART has been shown to result in a set of previously unrecognised toxicities, including kidney disease and osteoporosis. Both HIV infection and ART can independently affect the musculoskeletal system (Rosenfeldt

*et al*, 2005; Maagaard *et al*, 2006) and are associated with increased fracture risk (Triant *et al*, 2008).

A more in-depth discussion of ART is given in Chapter 2.

#### 1.2.2.4 HIV infection, ARV therapy, and bone health

In the past decade, osteoporosis and osteopaenia have been reported in HIV-positive populations in Europe, North and South America, Asia and Australia (Brown *et al*, 2006b). There have been a plethora of case reports and cross-sectional studies associating HIV infection with premature bone loss and symptomatic bone disease (Carr *et al*, 2001; Moore *et al;* 2001, Allison *et al;* 2003, Amiel *et al*; 2004, Paccou *et al*, 2009). In 2005, the estimate of osteoporosis prevalence in HIV-infected adults, based on a meta-analysis of aBMD, was as high as 15% (Brown *et al*, 2006b). However, some earlier reports, such as those of Paton *et al*, suggested that HIV infection was not associated with low aBMD (Paton *et al*, 1997; Bolland *et al*, 2006). In addition, there is some evidence to suggest that ART exposure may accelerate HIV-associated bone loss (Brown *et al*, 2009). There have been few reports of fracture incidence, but there is evidence from two studies that HIV-positive patients are at increased risk of fracture, in part because of low aBMD, compared to controls (Triant *et al*, 2008; Yong *et al*, 2011).

Numerous cross-sectional, and some longitudinal, studies have illustrated the negative effects of ART exposure on bone health. The effects of ART have also been demonstrated in HIV-negative individuals exposed to ART as part of pre-exposure prophylaxis (PrEP) trials to prevent HIV infection. This provides powerful evidence that tenofovir (a commonly prescribed NRTI), at least, has an effect on bone mineral separate from other ART, and indeed HIV infection per se. Not all studies demonstrate a negative effect of ART on bone health and for those that do the effect of different classes of ART, and indeed individual drugs within these classes, on bone health remains to be fully elucidated.





The available data on bone health in the context of HIV infection and ART exposure are limited because studies have focused largely on younger adults, who have, historically, been most affected by HIV but least affected by osteoporosis. In addition, data on aBMD in HIV-infected patients are mainly from young males. This makes extrapolation to women and older people difficult since lower aBMD in young male patients may not be as predictive of subsequent fracture. In the meta-analysis by Brown and Qaqish of all studies of aBMD in HIV patients up to 2005, only 11 included HIV-negative controls, the majority of whom were male and, apart from one Argentinian study, all were conducted in high income countries (Brown *et al*, 2006b). Most of the studies evaluated were not longitudinal and therefore are not able to make the difficult distinction between effects of HIV infection and of ART exposure. Finally, HIV infection is associated with risk factors for osteoporosis, including abnormal gonadal function (Rietschel *et al*, 2000) and low levels of physical activity (Maharaj *et al*, 2011). These also need to be taken into account when assessing the effect of HIV infection and ART exposure on bone health as such risk factors may contribute more to decreases in bone mineral than HIV infection or ART exposure.

A more detailed critique of these studies and the potential mechanisms by which HIV and ART affect bone is given in Section 2.4.

### 1.2.3 Poor vitamin D status

#### 1.2.3.1 Epidemiology

Vitamin D deficiency causes rickets and osteomalacia in children, and osteomalacia in adults. Historically, these were regarded as diseases of temperate latitudes as a result of insufficient exposure to sunlight containing ultraviolet radiation B (UVB) for the skin to synthesise vitamin D (Holick, 2006; Schoenmakers *et al*, 2008). Improvements in air quality, which allowed more UVB radiation to reach the earth's surface, and fortification of foodstuffs with vitamin D, meant that classical rickets and osteomalacia became rare in the general populations of developed countries. However, there are certain groups of people, particularly those with darker skin and/or those who dress in a way that almost

completely covers their skin, who develop skeletal manifestations of vitamin D deficiency. In addition, it is now recognised that, despite abundant UVB-containing sunlight, rickets and osteomalacia can also occur in tropical countries (Schoenmakers *et al*, 2008).

A biochemical measure of vitamin D status can be used to ascertain if an individual or a population has sufficient vitamin D to meet requirements in order to prevent vitamin D-associated skeletal diseases. The most accepted measure of vitamin D status is via the measurement, in blood, of 25-hydroxyvitamin D (25(OH)D) concentration; this correlates with the skeletal diseases rickets and osteomalacia (outcomes) caused by poor vitamin D status. 25(OH)D has a relatively long half-life in the circulation and gives an integrated measure of vitamin D supply from endogenous synthesis and the diet. To date there is no single biomarker for vitamin D supply, function and risk of disease (Prentice *et al*, 2008). 25(OH)D, perhaps, provides the most consistent, reproducible, and affordable measure of vitamin D status, although research is required to better integrate these different aspects of vitamin D metabolism (Prentice *et al*, 2008).

Vitamin D status is determined by several factors including dermal manufacture of vitamin D when exposed to UVB. This is under the influence of several factors including atmospheric conditions, use of sunscreen or sun block, latitude, and skin covering and pigmentation. Other important contributors to vitamin D status are intake of vitamin D through diet and supplement use, hepatic and renal capacity to metabolise vitamin D, and tissue requirements for vitamin D.

The optimum UVB sunlight exposure for vitamin D production, vitamin D intake, and the use of markers of vitamin D status at a population level are controversial areas and the subject of much debate. The thresholds of 25(OH)D used to assess status, and define sufficiency, insufficiency, and deficiency, vary depending on the condition being described. For example, it is well established that 25(OH)D concentrations of less than 25nmol/l are associated with increased risk of developing rickets and osteomalacia. Poor





vitamin D status has also been linked with osteoporosis, fracture, and increased risk of falls (Ross, 2010). Case-controlled, cohort, and ecological studies have related higher 25(OH)D concentrations with reduced risk of bone fracture, falls, autoimmune diseases, type 2 diabetes, cardiovascular disease, and some malignancies (Wang, 2009). It has also been suggested that non-skeletal conditions may require greater 25(OH)D concentrations to prevent or reduce the risk of disease outcomes (Ross, 2010; Heaney *et al*, 2011). These controversies are examined in greater depth in Chapter 2.

#### 1.2.3.2 Vitamin D status and osteoporosis in HIV infection

While it is known that poor vitamin D status can result in adverse musculoskeletal outcomes in the general population (Scientific Advisory Committee on Nutrition, 2007; Ross, 2010; Bischoff-Ferrari *et al*, 2012), data are lacking around the long term effects of poor vitamin D status on the risk of poor bone health, including osteoporosis and fracture, in HIV-infected adult patients. It is as yet unclear if the HIV-positive population will mirror the general population in terms of disease risk associated with poor vitamin D status, or if this risk will occur at higher concentrations of 25(OH)D.

It is also unclear if the poor vitamin D status described in HIV-positive individuals is related to lifestyle factors (such as decreased time spent outdoors), problems with dermal synthesis of vitamin D, or the effects of the virus on vitamin D metabolism and turnover. Also, the chronic pro-inflammatory milieu associated with HIV infection may result in greater demands for vitamin D as a result of greater breakdown in the liver and kidney. Both HIV infection and ART exposure have the potential to affect each of these determinants of vitamin D status.

#### 1.2.3.3 HIV, ARV therapy, vitamin D status, and bone health

Many epidemiological studies have reported associations between HIV infection, with and without ART exposure, with poor vitamin D status and some report an inverse relation between vitamin D status and morbidity and mortality (Sudfeld *et al*, 2012). In such studies vitamin D status was generally assessed by the measurement of 25(OH)D or proxy of status by perturbations of calcium and bone metabolism, usually by measuring

plasma parathyroid hormone (PTH) concentrations or bone turnover markers (Aukrust *et al*, 1999; Bolland *et al*, 2008; Fabbriciani *et al*, 2011; Conesa-Botella *et al*, 2012; De Socio *et al*, 2012; Kwan *et al*, 2012).

HIV infection could have a detrimental effect on vitamin D status through many mechanisms, including resulting from general debility, with less time spent outdoors, poor appetite, and therefore less dietary intake, as well as the use of medications that induce the metabolism of vitamin D.

Some studies have found associations between specific ART and low plasma 25(OH)D concentrations (Conesa-Botella *et al*, 2010; Rosenvigne *et al*, 2010; Welz *et al*, 2010; Dao *et al*, 2011) while others did not (Stein *et al*, 2011). Other studies have reported different effects of Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI) and Protease Inhibitor (PI) medication-exposure on vitamin D status with NNRTI-use more consistently associated with poor status than PI-use (Kwan *et al*, 2012) (see section 2.1.2).

The association of HIV and ART-use with poor vitamin D status suggests that this may be a factor in promoting HIV-associated osteoporosis, and vitamin D deficiency is regarded as a secondary risk factor for low aBMD generally (see Figure 2-23). However, most data describing low aBMD and poor vitamin D status in the context of HIV infection are from cross-sectional studies that have lacked a HIV-negative control group, and/or studies in Caucasian males. In addition, other risk factors for osteoporosis need to be considered. These include 'traditional' (age, female gender, low body weight, smoking, high alcohol intake), 'HIV-related' (duration of HIV infection and ART exposure, viral factors, a pro-inflammatory milieu) and 'secondary' factors (renal dysfunction, acid-base disturbance, hypogonadism) (Walker-Bone, 2012). An added complexity is that there are overlaps between these categories: for example, renal dysfunction as a risk factor for bone loss may be the consequence of a combination of traditional (e.g. diabetes) and HIV-related (via HIV-associated nephropathy (HIVAN)) factors. In addition, poor vitamin





D status may be linked to sub-optimal immune system functioning, which might accelerate HIV-disease progression.

Greater detail about vitamin D metabolism and function in general and in relation to HIV infection and ART exposure will be given in Section 2.3.

## 1.3 The situation in South Africa

Accurate prevalence rates for low aBMD and low 25(OH)D status in urban South African women with or without HIV infection are unknown.

### 1.3.1 Osteoporosis prevalence

As previously described, there are limited reference data on the incidence and prevalence of osteoporosis in South Africa. The lifetime risk of whites, Asians and mixed-race populations in South Africa is thought to be similar to those of populations in the developed world, where there is a lifetime risk of 30-40% in women and 20% in men; "no accurate (South African) fracture data exist" (Hough, 2010). Hip osteoporosis is less prevalent in South African blacks than in whites, although vertebral aBMD, and possibly fracture risk, seem to be similar. The National Osteoporosis Foundation of South Africa set as one of their key recommendations, "local research on the incidence of risk factors for, and normal reference data on, osteoporosis" (Hough, 2010).

### 1.3.2 HIV prevalence

The most recent UNAIDS estimates (2011) reveal that South Africa had 5.6 million people living with HIV. Of the 5.3 million adult cases, 3.3 million were women. South Africa also had 314,000 HIV-related deaths in 2008. In 2009, overall HIV prevalence was 17.8%, the fourth highest in the world after Swaziland, Botswana and Lesthoto, and, in 2010, its ART coverage was estimated to be 55% (Motsoaledi, 2010; UNAIDS, 2011).

### 1.3.3 ART practice

In the UK, it is currently recommended that ART commences in uncomplicated HIV infection when blood $CD_4$ count falls to $\leq 350 \times 10^6$ cells/l (see Section 2.1.1). In South Africa, the corresponding $CD_4$ count threshold for ART initiation was $<200 \times 10^6$ cells/l until August 2011 when it was raised to match European/UK standards. First-line ART in

South Africa is based on a NNRTI regime and access to second-line therapies (see Section 2.1.2) is restricted in many public clinics.

### 1.3.4 Vitamin D status

South Africa lies in the latitude band 20-40$^0$ S (Prentice *et al*, 2009a). There are latitude, climatic, and cultural differences between the north and south of the country that affect the potential for dermal vitamin D synthesis. As a result, there are marked variations in vitamin D production in different areas and during different seasons. For example, Johannesburg (North East) (26$^0$ S) has greater peaks of in vitro vitamin D production in the winter when compared with Cape Town (South West) (35$^0$ S), but little difference in the summer (Pettifor *et al*, 1996).

There is a dearth of data regarding vitamin D status in Sub-Saharan Africa generally and South Africa. A review of African and South African studies describing vitamin D status was conducted by Prentice *at al* 2009 (Prentice *et al*, 2009a). In this review, nine South African studies in children, adults, and pregnant women were described. The mean 25(OH)D concentration was above 50 nmol/l in all except children with rickets and patients with hip fracture (Prentice *et al*, 2009a). Other review articles have reported similarly good vitamin D status in African populations (Lips, 2010; van Schoor *et al*, 2011). More recently, Kruger *et al* have described vitamin D status in urban and rural postmenopausal South African women with mean 25(OH)D concentrations well in excess of 50nmol/l, and there was no difference when these figures were corrected for HIV-status (Kruger *et al*, 2011). The value of 50nmol/l is referred to as it is the cut off identified by the Institute of Medicine (IOM) above which skeletal effects of vitamin D are unlikely to occur (see Section 2.3.6). High rates of obesity in South Africans may adversely affect vitamin D status by facilitating sequestration of 25(OH)D into adipose tissue thereby limiting its bioavailability (Wortsman *et al*, 2000). This potential for vitamin D to be sequestrated in adipose tissue will be reviewed in more detail in Section 2.3.





### 1.3.5 Studies of HIV-associated bone loss in South Africa

To date, there are no published studies specifically examining HIV-associated bone loss in South Africa, although data exist on related matters, such as the relationship between vitamin D status and PTH in South Africans, which do not specifically address HIV-status. There are unpublished observations of 11% incidence of osteoporosis in HIV-positive, postmenopausal black South African women (Conradie 2008, cited in Kruger *et al*, 2011). Data do exist for bone fractures in the general population: 31/100,000 in South African black women compared with 402/100,000 in African Americans (Anderson *et al*, 1994). The women in Kruger *et al*'s study reported a less than 3% 'incidence' of fracture, although this is difficult to interpret because the study design was cross-sectional and is likely to represent ever fractured rates rather than annual incidence (Kruger *et al*, 2011).

Among published studies of HIV-associated bone loss in the low and middle income countries, an Argentinian study, Bruera *et al*, with a small number of controls (n=31) compared to HIV-positive (n=111) subjects, demonstrated a lumbar spine T-score of greater than -1 in 58% of controls compared to between 35-54% of HIV-positive subjects, depending on ART regime ($p<0.01$). At the femoral neck site the figures were 85% and between 19-31%, depending on ART regime, respectively ($p<0.01$) (Bruera *et al*, 2003). More recently, a Senegalese study reported lower aBMD in HIV-infected adults (n=204: 134 women and 73 men) compared with controls (n=207 matched), using quantitative ultrasound (QUS) of the calcaneus. Adjusted analysis demonstrated a QUS BMD difference: -0.27 SD, 95% confidence interval (CI): -0.53;-0.002, P=0.05. Differences in BMI explained a third of the difference in QUS BMD (Cournil *et al*, 2012).

## 1.4 Plan of research

HIV infection, ART exposure, osteoporosis, and poor vitamin D status are interconnecting conditions each likely to negatively influence bone health. This is especially true for those with predisposing risk factors for low aBMD and bone loss in adulthood, such as advanced age, gonadal dysfunction and concomitant limited physical activity, all of which are seen in HIV-positive adult patients. Whilst there has been a concentration of

research in HIV-associated communicable diseases (e.g. TB) in South Africa and the effects of ART on these, insufficient attention has been given to the future health problems such as osteoporosis that may result from long-term ART exposure.

My own clinical observations of poor bone health and poor vitamin D status as a consequence of symptomatic disease in HIV-infected patients in inner city London informed my decision to undertake research into the relationship between HIV infection and bone health. I have combined this interest with a career-long engagement with global health issues. Because the burden of HIV infection is disproportionately heavy in the developing world, particularly in Sub-Saharan Africa, this is the region that will also shoulder the burden of HIV- and ART-related bone disease, should it exist or develop in the future. Nevertheless, virtually all studies on bone health and vitamin D status in adult HIV-positive populations have been carried out in Caucasian male subjects, and female and non-white subjects are therefore vastly underrepresented. For this reason, I designed a study that addresses the potential contribution of HIV infection and ART exposure to bone health and vitamin D status in black South African women. The aims and objectives of this study are specified below.

### 1.4.1 Summary of the study design

Premenopausal, urban, black South African women were recruited to take part in the study. Premenopausal women were chosen in order to avoid the confounding by rapid, menopausal bone loss. HIV-positive women with preserved $CD_4$ counts, anticipated not to require ART-initiation for the first 12 months of the study, were compared with those with low $CD_4$ counts who were due to commence ART soon after the baseline study visit (see Section 2.1.1). It was anticipated that this latter group would be exposed to ART-treatment for the vast majority of the study duration. These groups were defined to try to separate the effects of chronic HIV infection (Positive preserved $CD_4$: Ppres) from those of ART exposure (Positive low $CD_4$: Plow). HIV-negative (Negative reference: Nref) women were recruited to act as a reference group. The study used a longitudinal design to detect changes in aBMD and vitamin D status over 12 months. Subjects attended for





study visits at baseline, 6 and 12 months. At each visit anthropometry, body composition, and bone imaging was conducted, and blood and urine samples collected for laboratory analysis. A 12-month follow-up period was chosen as it was unlikely that significant changes in aBMD would be detectable at less than 12 months, and to account for seasonal variation in vitamin D status. A *post hoc* retrospective division of subjects into those who were ART-unexposed and –exposed was undertaken to better explore the relationships between ART exposure and body composition, bone and vitamin D status variables (Chapter 8).

There are approximately one million people receiving ART in South Africa, and this number is likely to rise significantly over coming years. Once commenced, the majority of individuals will continue ART lifelong. The attributable risk for bone health and vitamin D status is likely to be significant, even if only a modest effect was found.

## 1.5 Aims

The aims of the study were:

1. To compare baseline aBMD and size-adjusted BMC (SA-BMC) in HIV-positive women (Ppres and Plow), and non-HIV-infected controls (Nref);
2. To compare baseline body composition in Ppres and Plow, and Nref;
3. To compare baseline serum concentrations of 25(OH)D in Ppres and Plow, and Nref;
4. To evaluate the effects of HIV and ART on body composition (DXA), aBMD (DXA), bone strength (pQCT) in ART-naïve and -exposed, compared with controls at 12 months after baseline;
5. To evaluate the effect of ART on serum 25(OH)D in those on and off ART, compared with controls at 12 months after baseline.

## 1.6 Objectives

The objectives were to discover and describe whether, in black South African women:

1. HIV infection is associated with low aBMD and SA-BMC;

2. HIV infection is associated with alterations in body composition and fat:lean mass proportions;
3. HIV infection is associated with low 25(OH)D concentrations;
4. ART exposure is associated with decreases in bone mineral;
5. ART exposure is associated with decreases in 25(OH)D.

These hypotheses were tested to determine if HIV-associated bone loss and low 25(OH)D are prevalent in black, urban South African women and to allow quantification of the magnitude of any effects and potential clinical relevance. Of particular interest was the comparison of bone change and/or loss at predominantly trabecular and cortical sites (Bonnick *et al*, 2006) to demonstrate if HIV infection or ART exposure resulted in preferential change at different sites, as this could predispose to particular patterns of osteoporosis and fracture.

Women were chosen for this study on the following grounds:

1. They are at greatest lifetime risk of osteoporosis and fragility fractures;
2. Approximately 2/3 of those diagnosed with HIV infection in South Africa are women;
3. Women in South Africa generally acquire their HIV infection at an earlier age than men and therefore are likely to experience a longer duration of infection and ART-exposure with attendant effects on skeletal health;
4. Women are underrepresented in studies of HIV-related bone loss;
5. To include both sexes would mean that the study would have to be expanded and go beyond the confines of a PhD project.

### 1.6.1 Approach taken to address objectives/ test hypotheses

The questions that arose from the aims and objectives were evaluated by using DXA to measure aBMD; DXA and anthropometry to measure body composition, and serum 25(OH)D concentrations to determine vitamin D status. The investigation of change in these parameters, was addressed by arranging longitudinal follow up of HIV-positive and





HIV-negative women. Subjects were assessed at baseline (time 0) and followed up six and 12 months afterwards. Full details are provided in the study design, Chapter 3.3.1. In order to control for differences in season, and in particular seasonal differences in vitamin D status, the study was designed so that the participants were seen at baseline and 12 months during the summer and autumn months and at the six month visit during the winter and spring months.

At each time point, anthropometry, DXA scans, and fasting blood and urine samples were collected in order to assess body composition and bone mineral measures and conduct vitamin D-, calcium- and phosphate-related laboratory analysis. Full details of the methods employed are described in Chapter 4.

## 1.7 Structure of thesis

Chapter 2: "HIV infection, bone health and vitamin D". This chapter provides a detailed overview of HIV infection, ART, bone biology and bone health, especially osteoporosis, and vitamin D metabolism and status.

Chapter 3: "Study design:" describes the design and the process of setting up the study in South Africa.

Chapter 4: "Methods." This chapter describes the methods used.

Chapter 5: "Baseline results": anthropometry, bone and body composition, socio-economic status, vitamin D status and biochemical analyses.

Chapter 6: "Cross sectional analysis." This chapter describes body composition, bone, vitamin D and biochemistry data cross sectionally at six and 12 months.

Chapter 7: "Longitudinal analysis". This chapter describes the *change* in body composition, bone, vitamin D, and biochemical variables over the duration of the study in the three groups.

Chapter 8: "*Post hoc* analysis of ART-unexposed and –exposed". This chapter describes the *change* in body composition, bone, vitamin D, and biochemical variables over the duration of the study in the subjects when divided into those unexposed and exposed to ART over the duration of the study.

Chapter 9: Discussion.





# 2 HIV infection, bone health, and vitamin D status

## 2.1 HIV/AIDS, a brief historical perspective

By March 1981, 8 cases of Kaposi's sarcoma, a rare cancer in young people, had been recorded in young gay men in New York City (Figure 2-1). At a similar time there were reports of sporadic cases of pneumocystis pneumonia (PCP), a rare form of the disease associated with profound immune suppression, reported in young gay men in California and New York. In June 1981, the Centers for Disease Control (CDC) reported 5 cases of PCP in men in Los Angeles; this report is sometimes termed the beginning of 'AIDS' awareness, although at that stage the term had not yet been coined. By July 1982, over 400 cases of PCP were reported to the CDC in gay men but, in the same year, doctors in Uganda had begun to recognise a condition associated with wasting in young adults that was termed 'slim' disease (AVERT, 2011), and when the syndrome was recognised in heterosexual Haitian patients later that month, it was no longer possible to continue classifying the condition as 'gay compromise syndrome', GRID (gay-related immune deficiency), or 'gay cancer'.

Doctors thought AIDS was an appropriate name because the condition was acquired rather than congenital; because it resulted in a deficiency within the immune system; and because it was a syndrome, with a number of manifestations, rather than a single disease (an anagram of AIDS (Acquired Immune Deficiency Syndrome), SIDA, was created for use in French and Spanish). But as Mann put it,

> "The dominant feature of this first period was silence, for the human immunodeficiency virus (HIV) was unknown and transmission was not accompanied by signs or symptoms salient enough to be noticed. While rare, sporadic case reports of AIDS and sero-archaeological studies have documented human infections with HIV prior to 1970, available data suggest that the current pandemic started in the mid- to late 1970s. By 1980, HIV had spread to all five continents (North America, South America, Europe, Africa and Australasia).

During this period of silence, spread was unchecked by awareness or any preventive action and approximately 100,000-300,000 persons may have been infected" (Mann, 1989).

In May 1983, researchers at the Pasteur Institute, Paris isolated a new virus thought to be the cause of AIDS, but by 1986 there was controversy over the name of the virus. There were two competing groups; the Pasteur group termed it LAV (Lymphadenopathy-Associated Virus) while Gallo's group in the USA called it HTLV-3 (Human T-cell Lymphotropic Virus, type three). In May of 1986, the International Committee on the Taxonomy of Viruses ruled that both names should be dropped and resolved the dispute by providing a new name, HIV (Human Immunodeficiency Virus). By the end of 1986, 85 countries had reported 38,401 cases of AIDS to the World Health Organization. By WHO region these were: Africa 2,323, Americas 31,741, Asia 84, Europe 3,858, and Oceania 395 (Bureau of Hygiene & Tropical Diseases, 1986).

A full account of the history of the HIV epidemic can be found at on the AVERT website (AVERT, 2011) and in Pepin (Pepin, 2011).

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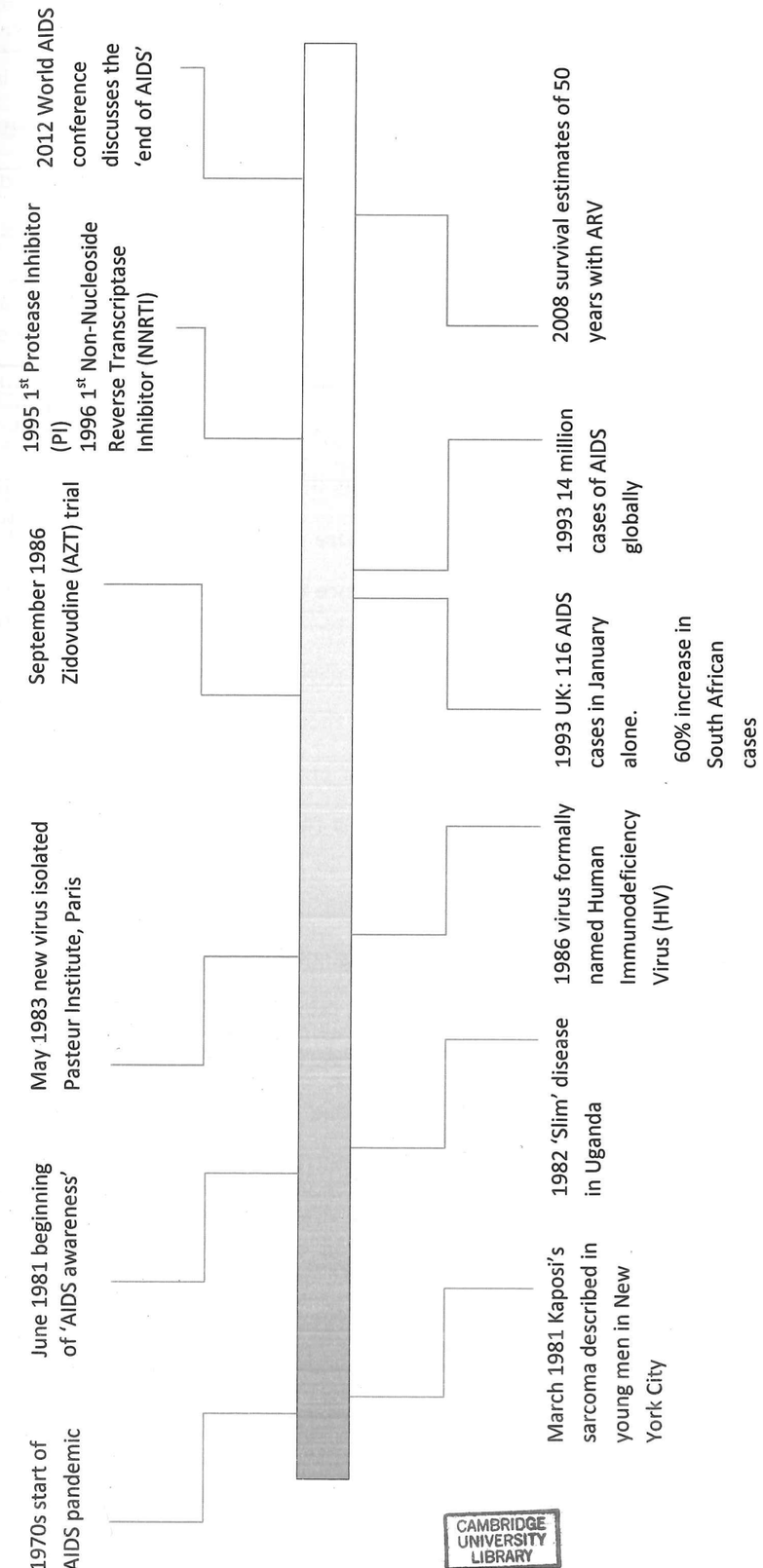


**Figure 2-1 HIV/AIDS timeline**

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### 2.1.1 HIV virology

HIV is a member of the genus lentivirus; a member of the Retroviridae family, and there are two different types, HIV-1 and HIV-2. HIV is thought to have evolved in humans from two distinct forms of Simian Immunodeficiency Virus (SIV): HIV-1 from chimpanzee-SIV, and HIV-2 from Sooty mangabey-SIV (Lemey *et al*, 2003). SIV probably crossed into humans through blood contact via hunting of monkeys and apes for food. HIV-1 and HIV-2 share approximately 40% of their genetic structure. However, the transmissibility is lower for HIV-2 and it is generally considered to be less pathogenic than HIV-1. HIV-2 can, however, cause AIDS that is indistinguishable from AIDS caused by HIV-1 and has a different pattern of susceptibility to ART. HIV-2 is largely restricted to West Africa with smaller numbers of patients in some southern European countries as a result of West African association. For a more detailed description see de Silva *et al* (2008, 2012). In this thesis any further reference to HIV refers to HIV-1 infection.

Three groups of HIV-1 have been identified based on structural differences in the viral envelope (*env*): M, N, and O. Group M is the most common and consists of 8 subtypes with distinct geographical variability. The most prevalent are subtype B (found mainly in North America and Europe), subtypes A and D (found mainly in Africa), and subtype C (found mainly in Africa and Asia).

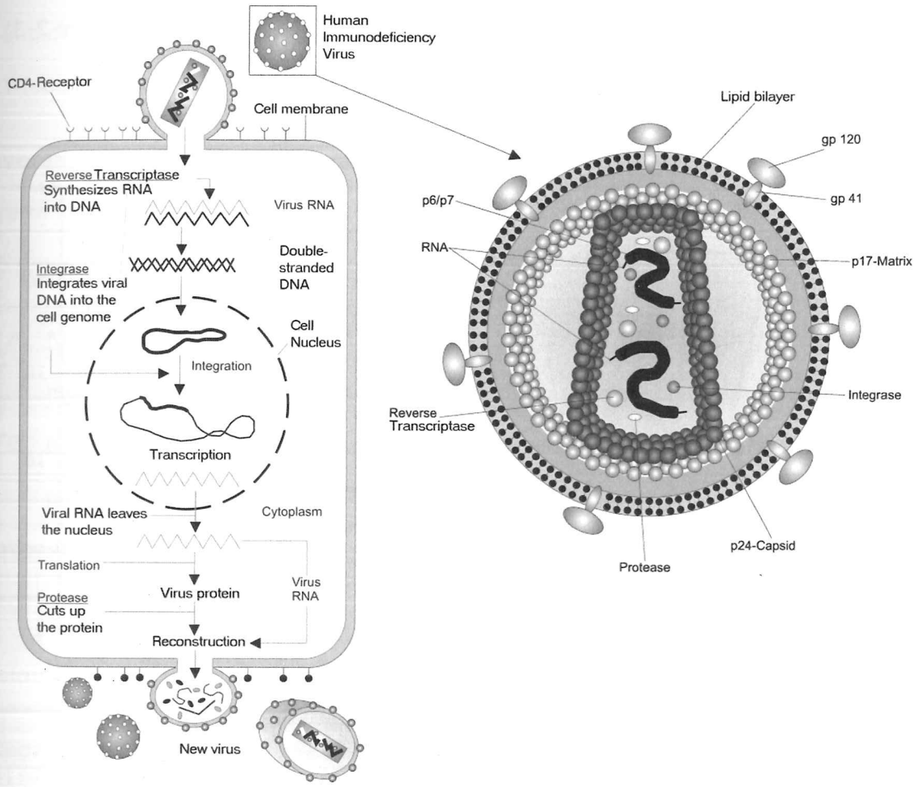
Lentiviruses are transmitted to host cells as single-stranded, positive-sense, enveloped RNA viruses. In the case of HIV the target cells are helper T cells (specifically $CD_4$-positive T cells), macrophages, and dendritic cells. When the virus enters the target cell, the viral RNA genome is converted into double-stranded DNA by the virally encoded reverse transcriptase enzyme. Reverse transcriptase is transported with the viral genome in the virus particle. The resulting viral DNA is then imported into the cell nucleus and integrated into the cellular DNA by a virally encoded integrase and host co-factors. Once integrated, the virus may become latent, allowing the virus and its host cell to avoid detection by the immune system. Alternatively, the virus may be transcribed, producing new RNA genomes and viral proteins that are packaged and





released from the cell into the blood stream as new virus particles that begin the replication cycle again (see Figure 2-2).

**Figure 2-2 HIV and life cycle**

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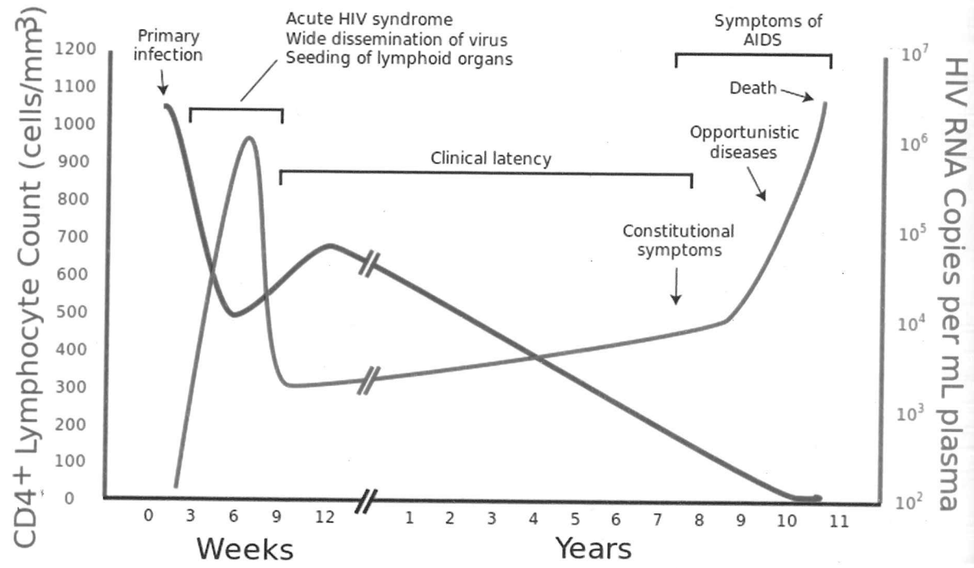
HIV infection leads to a destruction of target cells by means of three principal mechanisms. First, HIV is responsible for direct viral killing of infected cells; second infected cells undergo increased rates of apoptosis (cell death); and, third, infected $CD_4$-positive T cells are destroyed by $CD_8$-positive T cells, which recognise virally infected cells. The virus infects and destroys $CD_4$-positive T-lymphocytes causing $CD_4$ cell decline, usually over many years, eventually leading to $CD_4$ cell collapse (NIAID, 2010) with loss of cell mediated immunity. A 'normal' blood $CD_4$ count of a non-HIV infected person is in

the region of 500-1200 x$10^6$/l. When the $CD_4$ cell count falls below 200 x$10^6$/l, opportunistic infections begin to appear and tend to increase in number and severity as $CD_4$ levels continue to decline. The development of opportunistic infection (or HIV-associated malignancy or severe constitutional symptoms) indicates severe immune dysfunction and this is the stage of infection defined as AIDS. If untreated the prognosis for survival following an AIDS diagnosis is in the region of 18 months (see Figure 2-3).

**Figure 2-3 Natural history of untreated HIV infection**




(Sigve, 2013) [The person who associated a work with this deed has dedicated the work to the public domain by waiving all of his or her rights to the work worldwide under copyright law, including all related and neighbouring rights, to the extent allowed by law. See: http://en.wikipedia.org/w/index.php?title=File:Hiv-timecourse_copy.svg&page=1]

For information on the treatment of HIV infection, see section 2.1.2.

### 2.1.2 ART general

In parts of the world where there is ready access to ART, the life expectancy of those infected has improved. As a result, health can be restored and life expectancy improved by several decades. Estimates of prognosis of survival may be as great as 50 years, for a patient in their 20s diagnosed now with HIV infection, depending on nadir (lowest recorded) $CD_4$ count (The Antiretroviral Therapy Cohort Collaboration, 2008). Currently, there are 31 individual different drugs to treat HIV infection, and more being added each year, but none eliminate the virus. Different classes of individual ARV drugs are therefore taken in combination in order to disrupt HIV replication at different stages of its life cycle (Pieribone, 2003).In order for HIV to remain suppressed, ART has to be taken daily. Poor compliance with ART frequently results in the virus becoming resistant to one or more of the constituent ARV drugs. There are currently five different classes of ART (see Appendix 1).

**Reverse transcriptase (RT) inhibitors** interfere with the critical viral process of reverse transcription during which the viral enzyme RT converts its RNA into DNA. The two types of RT inhibitor are:

A. Nucleoside RT inhibitor (NRTI) – these are 'faulty DNA building blocks' that are incorporated into HIV DNA resulting in incomplete DNA chain formation;
B. Non-nucleoside RT inhibitor (NNRTI) – these bind to RT, interfering with the process of converting viral RNA into the required DNA.

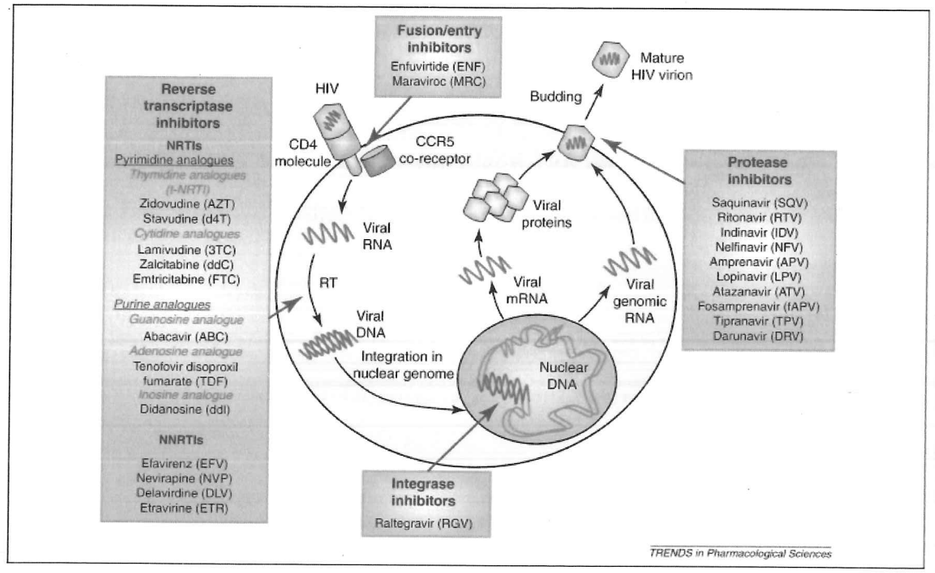
**Protease inhibitors (PI)** inhibit the viral protease enzyme that is central to forming infectious viral particles.

**Fusion and entry inhibitors** prevent viral fusion with the host cell membrane.

**Integrase inhibitors** block the viral enzyme integrase that allows viral genetic material to integrate with the host DNA (see Figure 2-4).

When a patient first requires ART, three drugs are generally combined to form highly active ART (HAART). Second-line ART is used when first-line ART is no longer effective in controlling viral replication. Second-line therapy is available in developing countries, e.g. South Africa but not always easily available (South African Department of Health: Motsoaledi, 2010). Fusion, entry, and integrase inhibitors are usually reserved for patients who have developed extensive viral resistance; they are extremely expensive and therefore not readily available in the developing world. Figure 2-4 illustrates the sites of actions of current ART.

**Figure 2-4 HIV life cycle and sites of ARV-drug actions**




(Apostolova *et al*, 2011) [Reproduced with permission]

The first reports of anti-HIV drug trials were in September 1986 using zidovudine (AZT) and reported the following year (Fischl *et al*, 1987). The effects on those using the drug were so markedly better than on the placebo group, in terms of improved survival, that the trial was halted early, even though AZT was associated with significant toxicity (Richman *et al*, 1987). But it was not until 1995 that a new class of ART was licensed; the protease inhibitors (PI) followed in 1996 by Nevirapine (a NNRTI). By 1996, a potent combination of ARV drugs was available, capable of reversing the devastating effects of AIDS, and the health of many improved enormously when they started taking this combination therapy. Some of those who had been ill in hospital were then able to go home, the improvement was so dramatic that it was referred to as the 'Lazarus Syndrome' (Andriote, 1999). From 1996 onwards, combination ART also meant that the mortality associated with HIV infection dramatically fell in the developed world. By 1998 there were, however, reports of abnormal fat-distribution (lipodystrophy) in HIV-positive individuals, particularly in association with PI-exposure. Toxic effects of tenofovir on the





kidney are also well established in clinical studies. They include phosphate wasting, acute renal injury and Fanconi syndrome (Fernandez-Fernandez *et al*, 2011).

### 2.1.3 HIV in South Africa

HIV infection is a major epidemic in Southern Africa, and the effect on morbidity, mortality, society, and culture cannot be overstated in countries where a large minority of the adult population are affected. Even in countries with relatively advanced medical infrastructure and free access to HIV-testing and treatment, such as South Africa, many individuals do not present to clinical services until they have advanced disease or AIDS. However, even at this stage, ART use can reverse symptoms and restore immune function and life expectancy. Nevertheless, where ART is available and where medical structures are in place to facilitate roll-out to the community, the shape of the epidemic is changing: young adults are no longer dying in vast numbers. Countries like South Africa have, recently at least, made great progress in increasing ART coverage to its population in a relatively short period of time. Indeed many HIV-infected patients are living long enough to develop non-communicable conditions such as obesity, diabetes, and hypertension.

It is therefore, in countries like South Africa, that overlapping epidemics of communicable and non-communicable disease are likely to be most evident because of the very large numbers of individuals affected by both. This is termed a 'double burden' of disease given the ageing population (and associated non-communicable disease) and high communicable disease prevalence (Mayosi *et al*, 2009; Chisholm *et al*, 2012; Marquez *et al*, 2012). In fact, in recent years, South Africa has described its population suffering from a 'quadruple burden' of disease, which includes maternal and perinatal, and injury-related conditions (Mayosi *et al*, 2009).

In 2000, there was increasing global criticism of the stance of the South African President, Thabo Mbeki, on HIV/AIDS. He was commonly described as being an AIDS denialist; that is, denying the link between HIV infection and AIDS. As a result, there

was a lack of a coordinated response to South Africa's burgeoning HIV-epidemic, which may have resulted in the loss of thousands of lives (Chigwedere *et al*, 2008; Chigwedere *et al*, 2010). In August 2001, AIDS activists took legal action against the South African Health Ministry over its continuing refusal to supply ART to prevent mother-to-child transmission (PMTCT) of HIV. In December of that year, it was ruled that the South African government should give pregnant women free access to the drug nevirapine. The judge ordered the government to set up a nationwide PMTCT programme with a deadline for an implementation report to be handed back to the court by March 2002. The initial commitment was to establish PMTCT services and, later, to increase ART coverage more generally in the population. However, the South African Government did not develop a comprehensive plan for widespread ART distribution until 2007 (AVERT, 2011).

## 2.2 Osteoporosis

### 2.2.1 Osteoporosis epidemiology

The term osteoporosis was introduced in France and Germany in the nineteenth century, based on histological observations of porous bone (Hillier *et al*, 1997). The main consequences of osteoporosis are fractures of the hip, vertebrae, and wrist. These fractures place a huge burden of morbidity and cost on societies with a high prevalence of osteoporosis. In the general population the incidence of bone fracture is bimodal with peaks in adolescence and the elderly. In young people the incidence of fracture is higher in males and the cause is more likely to be traumatic, but after the age of 35 years the overall fracture incidence in women increases dramatically so that female rates are double those of men (ibid.), and is more likely to occur with minimal trauma.

The major determinants of risk for osteoporotic fracture in older people, therefore, are increasing age and female sex. Age is also important because of its positive association with increased risk and rate of falling, so that by age 80 – 84 years a third of women in the USA will sustain at least one fall per year. Similar data available from the UK show that at an age greater than 85 years, 50% of women, and a third of men, will sustain at least one fall per annum. Despite this very high rate of falls it is estimated that only





approximately 1% of falls result in a hip fracture (ibid.), which however in absolute terms results in a very large incidence of fragility fractures. Men are relatively protected from osteoporotic fractures for several reasons; these include higher peak bone mass, less loss of bone mass with ageing, preserved gonadal function (compared to women), fewer falls, and the fact that they have a shorter life span in general (ibid.).

Osteoporotic fractures, particularly of the hip, may be devastating; such fractures lead directly to complications such as pressure sores as well as long term complications, including permanent impairment of ambulation in as many as 50% of those sustaining a hip fracture. Within 12 months of sustaining such fractures 5-20% will die, and the majority of those will die within 6 months of the fracture event. Mortality is, in part, dependant on age, with increasing age a risk for mortality (Beaupre *et al*, 2012). These figures are consistent in Western and non-Western settings (Yoon *et al*, 2011; Valizadeh *et al*, 2012). Osteoporotic fractures of the lumbar spine, and to a lesser degree the forearm, are also associated with significant morbidities (Hillier *et al*, 1997). These include pain, kyphosis and loss of height and associated respiratory symptoms, and are independently associated with increased risk of mortality (Hasserius *et al*, 2005). An important explanatory mechanism behind this increase in fracture risk is the decrease in bone strength, via decreases in bone mass, with advancing age. It is estimated, for example, that from its peak, femoral neck density declines by 58% in women and 39% in men after age 50 years (see Figure 2-11). The figures are similar for the intertrochanteric hip region, 53% and 35% respectively.

The epidemiology of vertebral fracture is more difficult to delineate because many of these fractures are occult and they have no universally accepted definition. Since many of these fractures are asymptomatic or associated with minor symptoms, patients are less likely to present to their medical practitioner and undergo a diagnostic x-ray examination. In general, the risk factors are thought to be similar for vertebral and non-vertebral fracture. However the incidence of vertebral fracture may be twice that of hip

fracture and this is important because the excess mortality is similar to that seen following hip fracture (Hillier *et al*, 1997).

Ethnicity, geography, and seasonality are also important factors in determining fracture risk. As outlined in Sections 1.2.1 and 1.3.1, white populations are more likely to sustain fractures than non-white populations. These ethnic differences have been explained by greater bone mass and density in non-whites compared to whites. However, this difference in bone mass and density is insufficient to explain all ethnic differences; for example, women of Japanese heritage have lower aBMD than their white counterparts, but their incidence of hip fracture is approximately 50% lower (Ross *et al*, 1991) and may, in part, be explained by differences in hip axis length. As studies in developing world populations continue to be published, the balance of evidence may show that these historical, ethnic differences may be more complex and nuanced than previously thought (Aspray *et al*, 1996; Yan *et al*, 2003; Aspray *et al*, 2005; Leslie, 2012). There are, moreover, very wide geographical differences in rates of fracture between, and even within, countries, and there are many epidemiological associations that have been suggested as explanations. In a US study, poverty and access to fluoridated water were positive correlates and southern latitude and water hardness were negative correlates of fracture rates (Jacobsen *et al*, 1990). Fractures are also seasonal, with increases in winter months. Since most fractures occur after falls indoors it is not likely that slips on ice and snow are the only primary causal factors. Other factors such as abnormal muscle functioning in cold weather and poor vitamin D status have been suggested. In contrast, the peak in distal forearm fractures seen in winter months is much more closely related to falls out of doors (Hillier *et al*, 1997). Future predictions of global fracture prevalence are difficult to estimate but appear to be set to increase dramatically, particularly in the developing world (Harvey *et al*, 2010).

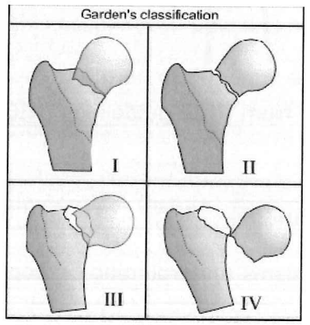
### 2.2.2 Osteoporotic hip fracture

The most important fragility fracture site is the hip; it has the greatest individual, health care-related, and societal costs. Osteoporotic hip fractures tend to occur following a fall



but can occur with other low impact trauma or even spontaneously. They cause pain and deformity of the affected limb and, in the developed world, almost always result in hospitalisation. In this acute setting, hip fracture is confirmed using X-ray examination and is classified depending on the anatomical location. Most are defined as intracapsular or extracapsular. The most commonly used classification system for intracapsular, or femoral neck fractures, is some variation of the Garden classification (see Figure 2-5 and Table 2-1). As its basis, the classification separates non-displaced fractures from displaced fractures because of the better rate of healing in the former.

**Figure 2-5 Garden's classification**




(Glasgow, 2012)

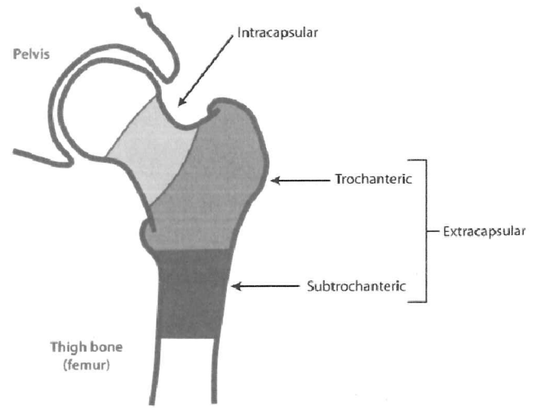
**Table 2-1 Garden's classification of intracapsular hip fracture**

| Stage | Description | Remarks |
|---|---|---|
| **Stage I** | Incomplete fracture of the neck | May be impacted and in valgus |
| **Stage II** | Complete without displacement | |
| **Stage III** | Complete with partial displacement | Fragments are still connected by posterior retinacular attachment |
| **Stage IV** | Complete femoral neck fracture with full displacement | Allows the femoral head to rotate back into anatomical position |

Extracapsular fractures are divided into trochanteric or sub-trochanteric depending on their anatomy; stability (stable or unstable) and displacement (present or absent) (see

Figure 2-6). These classifications are important as they often determine the choice of therapeutic intervention employed (Hillier *et al*, 1997).

**Figure 2-6 Schematic of intracapsular and extracapsular fracture**




Adapted from NICE guidelines (NICE, 2011)

### 2.2.3 Osteoporosis pathogenesis

The pathophysiological mechanisms underpinning osteoporosis are complex (Raisz *et al*, 2008). What can be briefly stated, however, is that an understanding of the pathophysiology of osteoporosis has changed perceptions of the disorder from "an inevitable consequence of aging … [to] … a disease of multifactorial aetiology for which an increasing number of therapeutic interventions are now available" (Compston, 2000). An understanding of the pathophysiological mechanisms allows for directed research into new therapies and treatments.

Genetic and environmental factors play important roles in the pathogenesis of osteoporosis since both affect peak bone mass and determine rates of subsequent age-related bone loss. The secondary causes of osteoporosis can be divided into five general areas: endocrine dysfunction, e.g. hyperparathyroidism; malignancy, e.g. lymphoma; drug use, e.g. corticosteroids; connective tissue disease, e.g. Marfan's syndrome; miscellaneous, e.g. chronic liver disease (Premaor *et al*, 2010). Despite this variety of





secondary causes, however, the majority of cases of osteoporosis are primary or idiopathic with no other cause identified (Compston, 2000).

In populations with adequate nutrition, bone mass accretion occurs during childhood and adolescence with rapid linear and appositional bone growth. By age 17 or 18 years the vast majority of peak bone mass has been achieved, although small increments may occur during the third decade of life (Compston, 1997). In other populations, such as those with delayed puberty (Naicker *et al*, 2010) this may occur over a longer duration. The onset of age-related bone loss is not precisely defined; a number of studies have discovered bone loss in premenopausal women and in men in the fifth decade (Compston, 2000). Women have an accelerated rate of bone loss following the menopause but the duration of this accelerated phase is not fully understood (Compston, 2000). In postmenopausal women, and men of 50 years and older, age-related bone loss continues for the remainder of their lives.

Raisz *et al* (2008) describe three "critical factors" that can lead to the development of osteoporosis:

1. A failure to achieve a satisfactory peak bone mass and strength. This is largely genetically determined but substantially affected by lifestyle;
2. Accelerated bone loss due to resorption. This is less dependent on genetic factors, with deficiencies in oestrogen, and calcium and/or vitamin D playing important roles;
3. An impairment of bone formation during remodelling. Soon after peak bone mass has been achieved there seems to be an inadequate capacity to form new bone to maintain skeletal mass during remodelling. The mechanism(s) underpinning this are not clear but are likely to include changes in local and systemic growth factor production and an accelerated absorption and impaired formation, driven by cytokine release.

#### 2.2.3.1 Menopausal bone loss

The adult female skeleton reaches peak bone mass by age 30 years and data on premenopausal bone loss are inconsistent. Age-related bone loss probably starts during the fourth decade of life and continues thereafter; it accelerates in women during the years around the menopause (Compston, 1997). Age of menopause and menopausal bone loss may be different in different populations. Changes in bone mass and calcium metabolism are evident during this 'perimenopausal transition'. Oestrogen is the most important hormone in terms of skeletal integrity although progesterone and ovarian testosterone may play a role (Reid, 2008). This may also have ethnically diverse patterns (Aspray *et al*, 2005).

Peak bone mass attained earlier in life is a major determinant of subsequent bone mass and fracture risk in later life (Compston, 1997). In contrast, Reid states that prior to the menopause transition there is virtually no bone loss in most regions of the skeleton and fracture rates are stable (Reid, 2008). The menopause "ushers in a period of bone loss that extends until the end of life and ...[is]... the central contributor to the development of osteoporotic fractures in older women" (Reid, 2008).

The rate and onset of bone loss varies by skeletal site. The greater metabolic activity and large surface area-to-volume ratio of trabecular bone mean that losses exceed those of cortical bone. It is estimated that 50% of trabecular and 35% of cortical bone mass is lost during the life time of an average woman, this compares with about a two-thirds loss of these amounts in men (Compston, 1997).

The most obvious effect of menopausal bone loss is the increase in fracture incidence (at the forearm and vertebrae, the trabecular rich sites of the skeleton), which is already demonstrable in the first decade following the menopause (Reid, 2008).

#### 2.2.3.2 Oestrogen deficiency

It is the oestrogen deficiency seen at menopause that plays a central role in the pathogenesis of postmenopausal bone loss (Compston, 2000; Raisz *et al*, 2008). It appears that oestrogen-induced bone effects are two-fold; by direct effects on bone cells





and indirectly via changes in the production of cytokines (e.g., TNFa) and growth factors by intramedullary cells (Compston, 2000). However the precise role of specific cytokines is unclear; that is, their pathophysiological role may not be definitively and directly linked to bone loss (Raisz *et al*, 2008).

Studies in osteoporosis have demonstrated an increase in Receptor Activator of NFκB Ligand (RANKL) activity (see Sections 2.3; 2.2.4.1). RANKL induces preosteoclasts to become mature osteoclasts and therefore results in bone resorption (see Section 2.2.4). RANKL concentrations rise in the context of oestrogen deficiency (Raisz *et al*, 2008) and so this may be one of the central mechanisms of oestrogen deficiency-associated bone loss. Even in women many years past their menopause, the small amounts of endogenous oestrogens still produced have a beneficial effect on aBMD. Hip and vertebral fracture rates are significantly higher in those with undetectable blood concentrations of oestradiol compared with those with measurable, albeit low, concentrations. These findings "challenge the ... belief that endogenous oestrogen production in postmenopausal women does not have physiologically relevant skeletal effects" (Compston, 2000). In men it is probable that the skeletal effects of testosterone are, in part, mediated by the conversion of testosterone to oestradiol, by aromatase, in bone (ibid.) and therefore, in part, accounts for decreased rates of bone loss compared to women.

#### 2.2.3.3 Osteoporosis and obesity

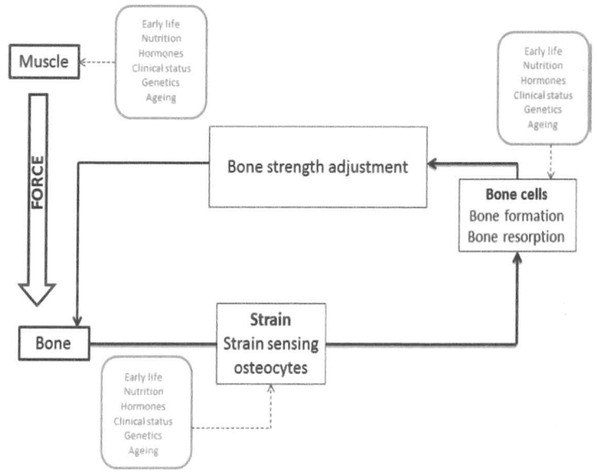
Obesity is usually associated with decreased fracture risk although this association may be changing with observations of lower leg fractures in obese individuals; in contrast decreased physical activity, which often accompanies obesity, increases risk of osteoporosis (Compston *et al*, 2011; Compston, 2013b). However the picture is mixed; there may be site specific pattern of fractures associated with obesity and fracture-associated morbidity may be increased but mortality may be reduced in obese compared to non-obese individuals (Compston, 2013a). How this combination of risk factors

translates into fracture risk in countries such as South Africa, with its increases in obesity and decreases in physical activity, remains to be seen.

The relatively new area of research of adiposity and bone health may provide clues into the possible future epidemiology of fracture risk in obese populations such as those in the UK and USA. Raisz *et al* (2008) frame the research in three ways.

1. That low 'body weight' is a clear risk factor for osteoporosis and that increased body weight has a positive effect on bone mass, though not necessarily on fracture (see Compston 2011, 2013). There is uncertainty about the relative roles of fat and lean mass in determining aBMD and it seems likely both play a role. A mechanism by which increased body mass affects bone is via mechanical loading as explained by the Mechanostat model (Ward, 2012) (see Figure 2-7). Also, high fat mass could increase oestrogen production via enhanced aromatase activity in adipose tissue.

**Figure 2-7 The Mechanostat**




A summary diagram of the Mechanostat model: the grey boxes indicate non-mechanical factors, including nutrition, and where they may interact with the Mechanostat. These interactions may be through alterations in the ability of muscle to generate forces (load), the bone ability to detect or respond to changes in load. For example, vitamin D causes proximal muscle weakness, which would reduce loading to bone and from this 'sub-optimal' bone accretion or increased bone loss may occur. At the bone level, vitamin D causes under-mineralisation of bone which would change the way it responds to loading. Both of these examples would result in changes in bone phenotype and strength.

(Ward, 2012) [Reproduced with permission]





2. 'Adipogenesis' in bone marrow increases with age and seems to be more pronounced in those with osteoporosis. Factors that promote adipogenesis may inhibit osteoblast formation, so decreasing bone formation (Webb, 2008).

3. 'Hormonal and neural factors' appear to play a role in determining bone mass. Sympathetic nerve fibres innervate bone and a number of neuropeptides act on bone, typically by increasing resorption. The observation that leptin deficient mice had high bone mass despite hypogonadism and that the leptin appeared to be acting centrally led to the postulation that the brain is involved in bone mass homeostasis (Raisz *et al*, 2008).

### 2.2.4 Bone biology and metabolism

The skeletal system is essential for normal bodily functioning; bone is one of its principal components and a metabolically active organ system responsible for vital functions. Bone is a "dynamic mineralised connective tissue with multiple physiological functions" (Grabowski, 2009). Three main functions are to provide the body with mechanical support, form a reservoir for calcium and phosphate, and to facilitate the production of haematopoietic cells by the bone marrow. Bone remodelling is the process by which old bone is removed and replaced by new bone as part of a coordinated process of skeletal maintenance that allows bone to respond to the body's requirements for calcium, phosphate, and acid-base homeostasis. Bones are dependent on other organs for their growth and maintenance, especially those of the gut and the kidneys through which minerals, such as calcium, are absorbed, reabsorbed and excreted, and vitamins, such as vitamin D, are metabolised. Other body systems such as skin, pituitary, muscle, fat, and gonads are important, directly or indirectly, for skeletal integrity (ibid.).

Macroscopically, bones can be described as either cortical or trabecular. Cortical, or cancellous, bone is the major bone type found in the shafts of long bones and is made of dense tissue penetrated by blood vessels and canaliculi that surround osteocytes. Trabecular bone is found at the end of long bones, near joint surfaces and in the

vertebrae; it consists of a network of thin plates and connecting 'struts' surrounded by bone marrow. Cortical and trabecular bone are similar in their cellular properties but differ in function and mechanical properties (Seeman, 2008; Grabowski, 2009; Anderson *et al*, 2012).

Bone tissue is made of a mineralised matrix (osteoid) produced by osteoblasts, which synthesise and mineralise the organic matrix, thereby laying down new bone. The collagen fibres of the osteoid matrix are mineralised by hydroxyapatite ($Ca_{10}(PO_4)_6(OH)_2$) to form a rigid structure. In addition to type 1 collagen, proteins such as osteocalcin and alkaline phosphatase (ALP) are secreted by osteoblasts into the organising matrix (Seeman, 2008; Grabowski, 2009). When their matrix-producing phase ends some osteoblasts undergo apoptosis while others differentiate into flat bone-lining cells that line the bone surface or become enclosed in the bone matrix in lacunae (osteocytes). In adults, 80% of trabecular and 95% of intracortical bone surfaces are covered by lining cells. The remaining surfaces are populated by osteoblasts and osteoclasts (Aubin *et al*, 2002).

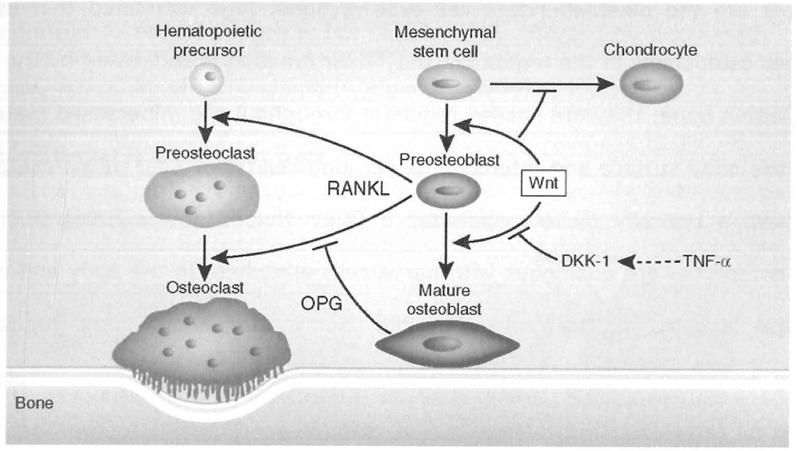
Osteoblasts, osteocytes, and bone-lining cells arise from a multipotent precursor of mesenchymal origin (Figure 2-8), which also gives rise to adipocytes and fibroblasts. In contrast, osteoclasts originate from a shared haemopoetic precursor lineage with macrophages.

Osteoblasts control the differentiation of osteoclast precursors. Osteoclasts are large, multinucleated cells found in close proximity to bone surfaces undergoing resorption. When apposed to bone, osteoclasts form a region of contact with the bone surface; this 'sealing zone' creates an area wherein bone resorption takes place via the action of secreted acid and proteolytic enzymes (Compston, 1997; Chen *et al*, 2000; Grabowski, 2009). Bone is, therefore, dynamic and undergoes constant formation and resorption, resulting in continuously modelled and remodelled bone. Bone remodelling depends on "the tightly integrated activity of two major cell types, osteoblasts and osteoclasts.



Therefore, the balance between the number and activity of osteoclasts and osteoblasts is crucial in determining bone mass, which is directly related to bone fragility and fracture risk" (Pan *et al*, 2006).

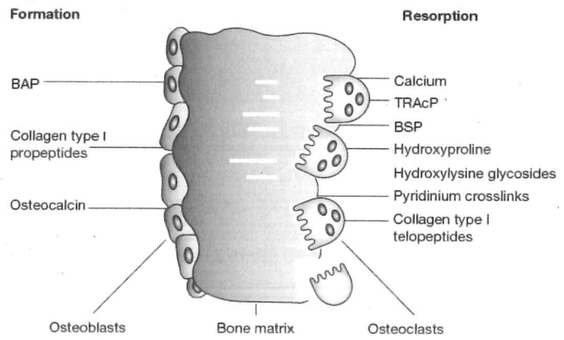
**Figure 2-8 Osteoclast and osteoclast pathways**




Wnt, Wingless-type MMTV integration site family, member 1; DKK-1, Dickkopf-1; TNFα, Tumour necrosis factor alpha; OPG, Osteoprotegerin. (Goldring *et al*, 2007) [Reproduced with permission]

Skeletal remodelling can be measured using a number of bone turnover markers (BTM) as depicted in Figure 2-9; markers of bone formation include ALP and osteocalcin and markers of resorption include collagen type 1 telopeptides (CTx).

**Figure 2-9 Schematic of bone formation and resorption**




BSP, Bone sialoprotein; TRAcP, Tartrate-resistant acid phosphatase (Seibel, 2005)

When rates of resorption are greater than formation then the bone matrix is disrupted and bone mass decreases and fracture risk increases. It is speculated that HIV infection has the effect of 'uncoupling' the normal formation:resorption process (Paccou *et al*, 2009).

Osteocytes are the most abundant cell type in bone. It is estimated that osteocytes outnumber osteoblasts in the region of 10:1. Their function is suggested by the strategic position within bone; they are spaced regularly throughout the mineralised matrix rather than on the bone surface and interconnect via long cell processes, within the canaliculi, giving them a typically stellate appearance (Klein-Nulend *et al*, 2008). Klein-Nulend asked if osteocytes are analogous with nerve cells elsewhere in the body and can sense mechanical loading and translate this into biochemical stimuli that regulate bone modelling and remodelling (Compston, 1997; Klein-Nulend *et al*, 2008). Osteocytes are also seen as central to the homeostasis of calcium and phosphate (see Section 2.3.8) (Klein-Nulend *et al*, 2008) and may be one of the target cells for the anti-resorptive bisphosphonate drugs (Chapurlat *et al*, 2000).

#### 2.2.4.1 Cellular pathophysiology of osteoporosis

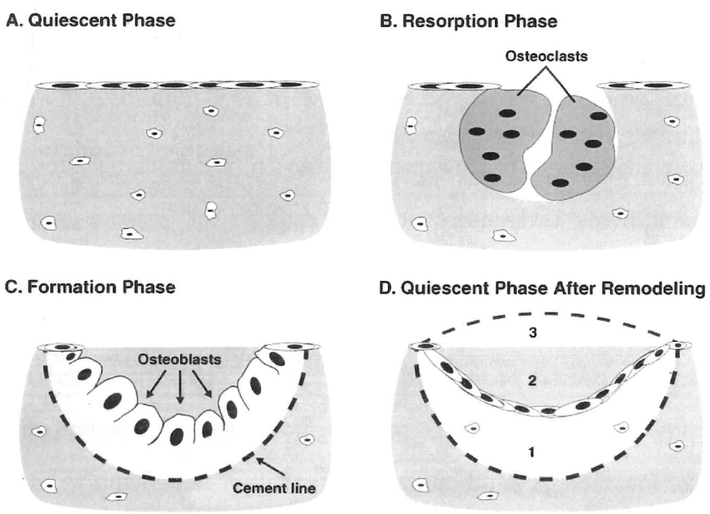
At a molecular and cellular level, two important processes influence bone loss. The most important, in terms of quantity, is increased bone turnover that is facilitated by an increase in number of activated remodelling units on the bone surface. The result is an increase in bone remodelling units undergoing resorption at any given time. The second process is the down regulation of bone formation within individual remodelling units, so that there is a 'remodelling imbalance' whereby bone is lost because of increased resorption, decreased formation, or a combination of the two (Compston, 2000) (see Figure 2-10).

It is these alterations in bone remodelling that determine the changes in cortical and trabecular bone architecture in osteoporosis. The deterioration in architecture is what decreases bone strength and increases fragility, hence fracture risk. The underlying mechanism by which strength is affected in trabecular bone is two-fold; bone loss is



associated with either trabecular thinning or penetration. Decreased activation results in trabecular thinning and increased activation (i.e., erosion depth or frequency) of the remodelling unit predisposes to penetration of the trabeculae (Croucher *et al*, 1996). These two states are intimately related as increased thinning will increase the likelihood of penetration. Trabecular thinning is associated with better maintenance of architecture than penetration so bone strength is not so adversely affected by decreased, compared to increased, activation of remodelling (Compston, 2000).

**Figure 2-10 Bone remodelling unit**




(Turner *et al*, 2001) [Reproduced with permission]

The main mechanism of post-menopausal bone loss is via increases in bone resorption. This process can be reversed by oestrogen or other antiresorptive agents such as bisphosphonates. These therapies improve bone density by filling in resorption cavities. At the level of the bone remodelling unit it is possible that both an increase in the depth of erosion and reduced wall width may contribute to bone loss. It is unresolved as to whether the pathophysiology of postmenopausal bone loss differs qualitatively from age-induced bone loss. Even postmenopausal bone loss cannot be viewed homogeneously in terms of bone turnover; Compston suggests that it may "represent the lower and higher

extremes, respectively, of peak bone mass and/or age-related bone loss in the normal population" (Compston, 2000).

The net effect of increased osteoclast activity, uncoupled from osteoblast bone formation is skeletal fragility (Raisz *et al*, 2008). The microarchitectural deterioration underpinning this probably reflects an increase in the number of osteoclasts formed and in their duration and intensity of action; RANKL can prolong osteoclast lifespan and oestrogen can decrease it. The interplay between different cytokines that may stimulate (e.g. TNFα, IL6) and inhibit (e.g. IL4) osteoclast function is very complex; they may influence each other so that a change in a single cytokine may not equate to its true physiological role *in vivo* (Raisz *et al*, 2008).

### 2.2.5 Bone health assessment techniques

The non-invasive assessment of bone health, strength, and mineralisation *in vivo* can be undertaken using many techniques including radiological imaging (e.g. radiographs, pQCT, ultrasound and DXA) to assess for presence of recent bone fractures, and for assessment of aBMD and bone age. Sampling of blood and urine can also provide important clues into overall bone health, particularly when the analytes measured in these body fluids give an indication of bone turnover (such as alkaline phosphatase) or concentration of metabolites and hormones essential for bone health and integrity (e.g. 25(OH)D and PTH).

DXA assessment is recognised as being clinically the most useful of the available bone assessment tools. It allows for the estimation of bone mass and density, and provides a basis on which to predict future fracture risk at clinically relevant sites. However, DXA measurement gives only a static view. In contrast, measures in biological samples can give a more dynamic assessment of bone health, which when used longitudinally can be used to measure change. Together, DXA-based and laboratory-based techniques give a more complete analysis of current bone status and possibly future bone outcomes (Section 2.2.3) than one technique alone.



These techniques are indirect measures of bone health. Direct analysis of bone structure and activity can be achieved by assessing bone biopsy material, for example with tetracycline labelling. However this is invasive and may not give a representative sample of the skeleton as a whole, rather the specific site of biopsy.

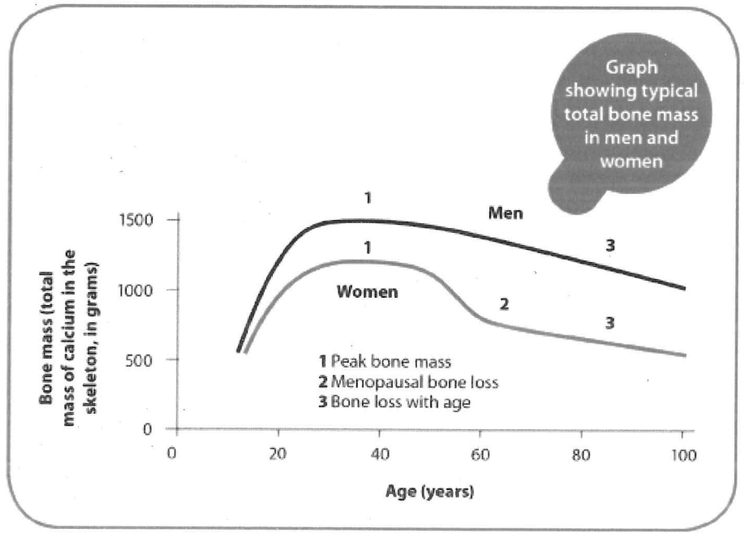
### 2.2.6 Measures of bone strength and determinants of fracture risk

In 1994 the WHO proposed four categories to describe aBMD and fracture risk, these are expressed in relation to the young, adult reference range (i.e. aBMD T-score) (see Section 1.2.1). They comprise of: i) normal, ii) low bone mass or osteopaenia, iii) osteoporosis, and iv) severe osteoporosis. These criteria were updated in 2008 and now specify that the reference site for diagnosis is the femoral neck. The young adult reference data are from the USA's NHANES III study based on young women aged 20 – 29 years with accompanying reference data for men (Hough, 2010).

#### 2.2.6.1 Peak bone mass

Bone strength is predominantly determined by bone mineralisation, which is a feature of attained peak bone mass (Figure 2-11), age-related bone loss, and total duration of bone loss; aBMD measured by DXA is a marker of bone strength.

**Figure 2-11 Typical bone mass and changes with age**




Arthritis Research UK (ARUK, 2012)

Peak bone mass is mediated by several factors including genetics, gender, body size, physical activity, and nutrition while involutional loss results largely from oestrogen deficiency (2.2.3.2), which increases bone resorption, and ageing, which is associated with decreased bone formation.

Bone strength is also mediated by the structural and functional properties of bone and overall bone quality. These can be separated into:

1. Macroarchitectural properties: bone size and geometry;
2. Microarchitectural properties: cortical thickness, cortical porosity, trabecular size, number and connectivity;
3. Bone turnover: i.e., dynamic between resorption and formation;
4. Material properties: collagen composition and cross linkage, mineralisation of collagen (primary and secondary) and repair of micro-damage.

These measures of bone quality are much more difficult to quantify than aBMD (Hough, 2010). A full discussion about risk factors for osteoporosis is beyond the scope of this thesis; a detailed examination is available (Abrahamsen, 2010). In brief, risk factors include lifestyle, genetic, nutritional, disease specific (e.g. of the endocrine system), age-related, toxic, qualitative factors (e.g. abnormal bone turnover) and a host of miscellaneous factors (Hough, 2010) (see Section 1.2.1).

### 2.2.7 Assessment of bone health by DXA

#### 2.2.7.1 What is DXA?

DXA imaging is a non-invasive x-ray technique and is an "extremely accurate and precise method for quantifying … BMD" (International Atomic Energy Agency, 2010). It allows differentiation of materials of different atomic mass (fat, lean and bone tissue) when they are in proximity to another material. This is possible because each material will have a unique X-ray attenuation at different energies (International Atomic Energy Agency, 2010). As X-rays pass from the source through the body, the physical properties of different tissues prevent the X-rays exiting the body to be detected by the X-ray detector (Figure 2-12). This process of attenuation is caused by the result of scatter,



that is, the deflection of X-rays away from the detector and absorption of X-rays into the body's molecules via ionisation (the transfer of electrons) (Webb, 2008). Denser tissues such as bone attenuate X-rays more than less dense tissue; heavier calcium and phosphorus atoms present in hydroxyapatite attenuate X-rays to a much greater degree than soft tissue. In 'soft tissue', protein-based lean tissue is denser than fat so attenuates X-rays to a greater degree (ibid.). This gradient of attenuation produces DXA images that are made from attenuation of low and high average X-ray energy. The X-ray densities used in DXA machines are optimised for bone density assessment.

DXA is accurate and precise in quantifying aBMD and body composition (International Atomic Energy Agency, 2010). DXA software has to quantify the amount of attenuation due to bone by correcting for the amount attenuated by lean and fat mass. It achieves this by projecting X-rays at two different intensities through the body. Different radiation energies produce a distinct pattern of attenuation depending on the composition of tissue through which it passes. To provide an estimation of attenuation the DXA software makes assumptions about the ratio of fat and lean mass overlying and next to the skeletal region of interest (Webb, 2008; International Atomic Energy Agency, 2010). The assumptions are made using soft tissue area that is adjacent to the area of interest so assumptions can be generated on the basis of bone and non-bone. In some regions, e.g. the trunk, it is not possible for the software to discriminate between muscle and abdominal organs. The combination of assumptions about fat and lean ratios with that produced by the attenuation of the two X-ray energies allows the software to produce attenuation values for fat, lean and bone. Any change in these proportions, e.g. as a result of a disease state, may result in inaccuracies in the quantification of bone. Attenuation constants for each tissue type are applied to give mass values present in each pixel (i.e., unit area). Bone mineral for a region, e.g., lumbar spine, is the sum of the values for each pixel in the region scanned (Webb, 2008; International Atomic Energy Agency, 2010).

DXA creates a 2-dimensional image that combines low and high density attenuations. However, it is limited by the fact that it quantifies areal rather than volumetric bone size and density. As a result, bone size is also important when interpreting DXA generated assessments of density, and size needs to be considered when interpreting measurement of mineral, especially when comparing individuals or populations of different size. Methods are available to try and minimise error caused by 2-dimensional measures of bone (Prentice *et al*, 1994). In addition, volumetric density can be measured using other imaging techniques, e.g. peripheral Quantitative Computed Tomography (pQCT).

The measure of radiation exposure is the 'effective dose' in Sieverts (Sv). This is generally used in preference to measures of absorbed dose in Grays (Gy) per kilo of tissue. This is because the effective dose controls for the susceptibility of different tissues to radiation damage and the type of radiation used. Effective dose is used in assessing occupational and public exposure to radiation. It is also useful in characterising the dose typically received by a patient from a given X-ray procedure (International Atomic Energy Agency, 2010) (Table 2-2). The radiation dose from DXA is low when compared with other general X-ray techniques, such as chest radiography, or bone-specific imaging techniques such as those required for the Singh index (see Section 2.2.7.2). It is rare for the radiation dose from a set of DXA scans for an individual to exceed 5μSV (0.005 mSv); this compares with a UK background daily dose radiation of 7μSv.

**Table 2-2 Typical effective radiation dose from diagnostic X-ray - single exposure (in mSv)**

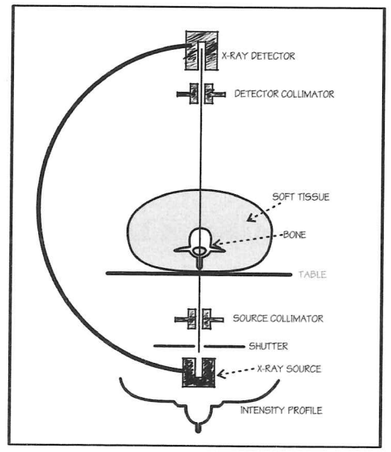
| **Examination; effective dose (mSv)** | |
|---|---|
| Chest radiograph | 0.04 |
| Pelvis radiograph | 0.7 |
| Lumbar Spine radiograph | 0.7 |
| Mammogram (four views) | 0.7 |
| Dental radiograph (panoramic) | 0.09 |
| Hip radiograph | 0.8 |
| Abdomen radiograph | 1.2 |
| Barium Enema (10 images, 137 sec. fluoroscopy) | 7.0 |
| CT Pelvis | 10.0 |
| Coronary Angiogram | 4.6-15.8 |

Adapted from 'Radiation exposure from medical diagnostic imaging procedures' (American College of Radiology, 2012)



There are three types of DXA system; pencil, narrow fan beam, and fan beam. Fan beam DXA scans require brief scanning time. For example, the time taken for a whole body scan is in the region of 6 minutes. The measurement 'precision' (%CV, that is the reproducibility of the scan) of DXA is also high with intra-machine CVs less than 3% (Mazess *et al*, 1990; Mazess *et al*, 1995) and accuracy (% difference from 'true' measurement) in the region of 3 - 8% (Blake *et al*, 1999).

**Figure 2-12 Schematic diagram showing the components of a DXA system**




(International Atomic Energy Agency, 2010) [Reproduced with permission]

The three bone values evaluated by DXA are:

**Bone Mineral Content (BMC):** the mineral component of bone i.e., hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$. It is expressed in grams (g) and does not include mass of the other organic components of bone such as marrow and collagen.

**Bone Area (BA):** the projected area of bone onto the image plane, it is expressed in $cm^2$.

**Areal BMD (aBMD):** the mineral mass of bone per unit image area in $g/cm^2$; aBMD=BMC/BA ($g/cm^2$) (International Atomic Energy Agency, 2010).

DXA also measures and calculates **lean mass (kg)** and **fat mass (kg)** and percentage body fat.

DXA is widely used in both clinical and research settings and normative values have been collated to ascertain an individual's aBMD, which can be used to diagnose low bone mass and evaluate the success of treatment of, e.g., antiresorptive therapy. For osteoporosis diagnoses, the lumbar spine, proximal hip, and sometimes the distal forearm sites are used when measurements at other sites are not appropriate (e.g. bilateral hip replacement). The whole body site is used to determine whole body bone mass and body composition i.e., fat and lean tissue.

The clinical and research utility of DXA in the diagnosis of low aBMD has several caveats; its role in diagnosising osteoporosis in children, men, and pre-menopausal women has not been clearly defined (Brown *et al*, 2006a) and normative data for developing world populations, and different ethnic groups in the developed world, are lacking. T-scores (1.2.1) therefore have limitations and need to take into account size, race, and gender (Prentice, 2004). Despite these limitations, DXA definitions of osteoporosis and fragility fracture risk have generally been accepted in all adult populations.

#### 2.2.7.2 Advantages of DXA

DXA is non-invasive, uses low dose radiation and is relatively quick to perform because of high photon flux from the X-ray tube. It is designed to measure important sites vulnerable to osteoporosis (hip and spine). It has been used to predict fracture such that, in postmenopausal women, the odds ratio for risk of hip fracture is 2.6 for every SD decrease in aBMD (Marshall *et al*, 1996). Therefore, DXA has utility as a diagnostic tool in identifying individuals at high risk of bone fracture. Whilst its precise role in fracture prediction has been disputed (Nielsen, 2000) it remains the 'gold standard' tool for assessing aBMD and fracture risk as defined by the World Health Organisation (WHO, 2003). The use of DXA to define osteoporosis in terms of T-scores <-2.5 is simple to interpret and prompts a set of clinical decisions to initiate investigation and treatment of low bone mass (Compston *et al*, 2009; Kanis *et al*, 2011).

DXA has clear advantages over other methods of assessing bone density. Other methods such as plain radiographs of the hip have been used in the assessment of bone; the



Singh index assesses trabecular patterns as an index for osteoporosis. However, these require X-rays of high resolution and therefore high radiation doses. Further, it is estimated that there needs to be a 30% change in trabecular pattern before a general diagnosis of osteopenia can be identified by visual inspection of radiographs.

#### 2.2.7.3 Disadvantages of DXA

DXA-associated radiation exposure is low; none-the-less it is additive to background radiation dose. Its use, and the pros and cons of scanning, have to be considered and only performed where justifiable in accordance with Ionising Radiation (Medical Exposure) Regulations (IRMER) guidance (Department of Health, 2000).

Whilst DXA aBMD results have been adopted to influence diagnostic and treatment decisions regarding osteoporosis, they do not fully account for differences in bone strength. It is estimated that differences in aBMD account for 70% of variations in bone strength and consequent fracture risk. Other factors, notably bone size and geometry, and the relative contributions of cortical and trabecular compartments play an important role (Laskey *et al*, 2010; Kanis *et al*, 2012; Li *et al*, 2013).

#### 2.2.7.4 WHO classification of low aBMD

While the WHO classification (2.2.3) provides a practical basis to identify those at increased fracture risk it is limited by the following factors:

1. A single aBMD assessment is specific (±85%) for predicting fracture but has poor sensitivity. As a result, less than half of those with a known osteoporotic fracture will have a aBMD T-score value of -2.5 or less (Wainwright *et al*, 2005);
2. WHO criteria are based on DXA data of the axial skeleton (i.e. spine and hip) from healthy postmenopausal women. Therefore extrapolation of these criteria to different populations, using different scanning techniques (e.g. QCT) of non-axial and/or axial sites is not correct;
3. The aBMD-based WHO diagnostic guidelines do not include non-skeletal risk factors (e.g. fall risk) or qualitative risks (e.g. bone turnover) which affect bone

strength. A low aBMD may result from metabolic bone disease other than osteoporosis, e.g. osteomalacia;

4. The four discrete diagnostic categories developed by the WHO should not be used to determine intervention (e.g. antiresorptive agents) in all cases (Hough, 2010) without reference to further guidelines (Compston *et al*, 2009).

There are also technical limitations to using DXA to provide information on bone area and density as measures of bone strength, and therefore using DXA to extrapolate information on bone strength and fracture risk may not be clear cut. There are several reasons for this including:

1. The bone volume is not known: DXA is a projectional technique and measures density in g/cm$^2$ as it is not able to measure tissue thickness. For this reason DXA cannot discriminate between small, high density bone and large, low density bone;
2. Fan beam magnification: Fan beams magnify BA via a factor proportional to the height of the scan table. Therefore, bone size can vary according to the body diameter (or body 'thickness'). Generally, there is a reciprocal demagnification in bone mass algorithms such that aBMD remains stable;
3. Two compartments: As mentioned above, DXA can only resolve 2 material densities simultaneously. Therefore soft tissue can only be resolved into fat and lean tissue in areas exclusive of bone whereas bone mass can only be determined by making assumptions about the proportion of fat to lean in the overlying soft tissues. Bone is contained in approximately 40% of the image pixels so soft tissue composition has to be estimated from that of neighbouring tissues. Where there is inadequate soft tissue outside of the bone projection (e.g., the head), manufacturers use proprietary methods to take account of soft tissue;
4. Standardisation: aBMD values generated by instruments from different manufacturers cannot generally be directly compared and are not interchangeable without cross calibration. There are several reasons for this including:



   a. Known differences in relative accuracy in aBMD; e.g., between Hologic and Lunar systems there are 8% and 20% differences in aBMD and BMC respectively because of differences between pencil versus narrow fan beam versus fan beam technologies, and different software assumptions.
   b. Lack of standardisation on placement of regions of interest (ROI).

   As a result, T-scores generated using different imaging modalities cannot be used interchangeably (Binkley *et al*, 2005; Kiebzak *et al*, 2007). Furthermore, the long term precision of individual devices should be considered.

5. Degenerative change: Degenerative change, vascular calcification, and fractured vertebrae may be difficult to visualise and cause significant, false, higher aBMD results. Disc degeneration and fracture reduce BA, increasing aBMD whilst calcification (especially in the aorta) projects more mineral into the pixels. These conditions increase with advancing age (International Atomic Energy Agency, 2010);
6. Inappropriate normative standards: Data outputted by different DXA systems may not be appropriate for the population studied. For example, using African-American data to generate DXA T- or Z-scores (Tothill, 1995) or Caucasian data using other imaging techniques (Laskey *et al*, 2010) for black Africans may be misleading and result in high rates of low aBMD. Reference methods such as the use of phantoms and analysis of porcine material post-scanning can provide some information but are not definitive for human comparisons (Tothill *et al*, 1994; Tothill *et al*, 1998);
7. Longitudinal study: Inter- and intra-individual results may be compromised even when using a single machine. This is pertinent to situations where weight loss or gain may be a feature because of assumptions made about distribution of non-bone material, especially fat (Yu *et al*, 2011). Animal fat (lard) to simulate weight gain was used in a study looking at the effects of gains in adipose tissue. The lard was placed over the subject's abdomen and the software was able to accurately measure the fat mass but also recorded an increase in BMC (Svendsen *et al*,

1993). Other factors such as menopause status (Movsesyan *et al*, 2003) and ART exposure may also influence fat mass;

8. Measurement error in DXA is equivalent to the average rate of bone loss per annum in peri menopausal women at a 1 - 3%. It is impossible therefore to discriminate if this magnitude of loss, should it occur, is due to 'real' bone loss or measurement error (Glüer, 1999; Shepherd *et al*, 2011);
9. Volumetric measurement: DXA calculates aBMD i.e., the mass of mineral per projected pixel. This does not equate to true density (=mass/volume), which is in fact volumetric (v) density. Given that it is impossible to measure a mass per volume using a projectional technique, aBMD is used as a proxy for vBMD. As larger individuals have larger bones, so they have higher BMC than smaller individuals (if aBMD is the same). This depth of bone is the dimension that is not corrected for by dividing BMC by BA to generate aBMD, and is why full correction for BA is essential for studies comparing individuals of different size.

#### 2.2.7.5 Adjusting DXA BMC and aBMD for size effects

Generally, data from absorptiometry are expressed in terms of areal measurements such as aBMD; these are derived by dividing BA by BMC in order to correct for size. This size correction assumes a linear and proportional relationship between BA and BMC when in fact this is not usually the case as the relationship depends on multiple factors including population being assessed and body size. Using a predefined index (BMD) may fail to fully correct BMC for skeletal size and lead to spurious associations of BMC with other size-related variables such as calcium intakes. In order to overcome some of these limitations, size adjusted-BMC (SA-BMC) has been calculated. This adjustment uses regression models to adjust BMC for BA, weight and height (Prentice *et al*, 1994). Such an approach does not detract from the importance of aBMD as a predictor of fracture risk but rather adds a further dimension for assessing differences in BMC between individuals by controlling for the effects of bone and body size on bone mineral status.





### 2.2.8 Osteoporosis assessment in non-Caucasian populations

One limitation of DXA is the difficulty of comparing low aBMD and fracture risk across populations. The majority of reference data are generated in developed countries and extrapolated to developing populations. Prentice has examined aBMD and subsequent fracture risk in different populations and demonstrated that aBMD is strongly influenced by body size (Prentice, 2004). Therefore, populations with traditionally shorter stature, who may be smaller and lighter, such as some African populations, "have lower bone mineral status than Western populations but do not have higher rates of osteoporotic fracture" (Prentice, 2004); this observation still is not fully understood. Nelson and colleagues have suggested a nuanced appraisal of various nutritional, anthropometric, geographical, dietary, and behavioural factors when comparing data and fracture rates across populations (Nelson *et al*, 2008). Whilst there is a paucity of data on osteoporosis in Sub-Saharan Africa, it is estimated that an increasing fracture risk is likely to be particularly prominent in the developing world with its adoption of a more westernised lifestyle (Harvey *et al*, 2010). This is likely to be important in a country such as South Africa, undergoing rapid nutrition transition with high rates of overweight and obesity (Mayosi *et al*, 2009; Hall *et al*, 2011) and decreasing levels of physical activity partly because of urbanisation.

Some African populations have been noted to have fewer fragility fractures compared to their Western counterparts than might be expected from aBMD measurements (Aspray *et al*, 1996; Zebaze *et al*, 2003). This may be due to differences in muscle strength, bone geometry and decreased propensity to falling in the elderly rather than simply a reflection of aBMD differences per se.

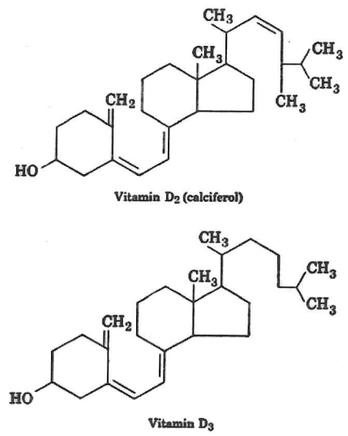
It is notable that bone data (and other risk factors for non-communicable disease), may also be significantly different between ethnic groups *within* a single country (Chantler *et al*, 2012) and therefore potentially problematic when compared *across* regions, e.g. diasporic Europeans or Africans, and Europeans or Africans residing in Africa (Rush *et al*, 2007).

## 2.3 Vitamin D

### 2.3.1 What is vitamin D?

Vitamin D is a group of fat-soluble secosteroids primarily responsible for intestinal absorption of calcium and phosphate. In humans, the most important related compounds of vitamin D are vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol) (Figure 2-13) and both can be ingested from the diet and/or supplements. The body can also synthesize vitamin D3 (from cholesterol) when UVB sun exposure is adequate. Therefore, although vitamin D is commonly called a vitamin, it is not an essential dietary vitamin in the strict sense, as it can be synthesized in adequate amounts by most mammals, including humans, exposed to sunlight.

**Figure 2-13 Molecular structures of vitamin D2 and D3**




Source: http://www.cyberlipid.org/vitd/vitd0001.htm

Vitamin D plays a central role in calcium homeostasis and skeletal health throughout the life course. As described in 1.2.3, it is a hormone that is essential for skeletal health; rickets and osteomalacia being the classical diseases of deficiency (Holick, 2008; Pettifor, 2008). There is some evidence that vitamin D is important in the pathophysiology of osteoporosis and non-skeletal disease and of potential adverse health outcomes at greater concentrations of 25(OH)D than seen in rickets and osteomalacia (Holick, 2007; Martineau *et al*, 2011; Coussens *et al*, 2012). The Institute of Medicine





(IOM 2010) reported that there is good evidence supporting the role of vitamin D (and calcium) in bone health, but evidence is lacking, especially from RCTs, in terms of non-skeletal health. The 2010 report stated that there is sufficient evidence to support the conclusion that an average blood level of 25(OH)D of above 20 ng/ml (i.e., 50nmol/l, (see section 1.3.4)) is needed for good bone health for practically all individuals (Ross, 2010) but there was not sufficient evidence to set blood levels with respect to non-skeletal disease.

Some authors challenge the IOM position on vitamin D status and predisposition to non-skeletal disease, as described above. There is controversy over definitions of vitamin D deficiency and insufficiency, the serum concentrations of 25(OH)D that equate to these terms and how this marker of status relates to disease outcome or risk (Heaney *et al*, 2011; Holick, 2011). It may be that higher thresholds need to be set in certain situations and relating to specific disease entities to define risk of these associated with these conditions.

### 2.3.2 Vitamin D epidemiology

The prevalence of clinical vitamin D deficiency is high in many parts of the world, including temperate and tropical countries, and all ethnic groups may be affected (Prentice, 2008). It is sometimes assumed that those living in tropical or subtropical countries will not have poor vitamin D status because of year-round UVB radiation but behaviours, habits, and other medical conditions can affect that ability to synthesise vitamin D in the skin. There has been a resurgence of vitamin D deficiency in Northern European countries, particularly in ethnic minority groups (Prentice, 2008). Risk factors for poor vitamin D status include prolonged exclusive breastfeeding, dark skin pigmentation, lack of direct sun exposure, residing in a northern latitude, and winter season (Huh *et al*, 2008). Poor vitamin D status can affect people at every stage of the life course, from foetal life through to old age (see Section 1.2.3.1).

### 2.3.3 Vitamin D production

It is estimated that in Western Europeans in the region of 90% of vitamin D is obtained from dermal synthesis, although this may be different in different populations (Prentice *et al*, 2008). In tropical climates, with year round exposure to appropriate UVB wavelengths, vitamin D deficiency is uncommon providing that the skin is exposed to sufficient sunlight. This is in contrast with temperate regions, such as the UK, where there are seasonal variations in dermal vitamin D synthesis. Dietary sources of vitamin D are largely limited to oily fish and fish oils, egg yolks and animal organs. Dietary supplements and fortified foodstuffs are important sources in some populations.

Solar UVB radiation of wavelength 290 to 315 nm (Olds *et al*, 2008) with an optimum wavelength thought to be 295-300 nm (MacLaughlin *et al*, 1982), penetrates the skin and converts 7-dehydrocholesterol to previtamin D3, in the epidermis (specifically in the stratum basale and stratum spinosum). Previtamin D spontaneously isomerises to cholecalciferol and diffuses out of the cells of the epidermis into the circulation. Vitamin D intoxication is avoided because any excess previtamin D3 or vitamin D3 is degraded by the action of sunlight. Therefore exposure to sunlight is a closed system preventing excess production of vitamin D. Since few foods naturally contain vitamin D (see above), synthetic vitamin D is manufactured commercially through ultraviolet irradiation of either 7-dehydrocholesterol in lanolin (D3) or ergosterol in yeast (D2) for use in supplements and fortified foods.

### 2.3.4 Vitamin D metabolism

The parent vitamin D compound is generally present in very low concentrations in blood and along with vitamin D from the diet, is transported to the liver by vitamin D binding protein (DBP). In hepatocytes, vitamin D is metabolised to 25(OH)D, facilitated by cytochrome P450 (CYP) pathways (CYP 2RI and 27AI) (Figure 2-14 ). 25(OH)D is secreted from hepatic cells into the circulation bound mainly to DBP. It is 25(OH)D that is used to assess a person's vitamin D status because it is a stable metabolite, present in nanomolar concentrations with a long half-life ($t_{½}$) of several weeks, which is relatively easily measured in blood. 25(OH)D acts as a reservoir and may undergo a further



hydroxylation step in the proximal renal tubule to its active form: 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), by the enzyme 25-hydroxyvitamin D-1-α-hydroxylase (CYP27B1), or to the catabolic form 24,25-dihydroxyvitamin D ($24,25(OH)_2D$). The renal production of the active compound is under very tight control by PTH, and calcium and phosphate concentrations as outlined in Section 2.3.8. $1,25(OH)_2D$ also induces its own degradation by stimulating expression of 25-hydroxyvitamin D-24-α-hydroxylase (CYP24), which catabolises 25(OH)D and $1,25(OH)_2D$ into inactive calcitroic acid (Holick, 2007). $24,25(OH)_2D$ also circulates bound to DBP and its production is an early step in the degradation of 25(OH)D thereby preventing intoxication. The fact that it can be found in significant amounts even in those with vitamin D deficiency suggests that it may have a distinct physiological role yet to be fully understood (Prentice *et al*, 2008).

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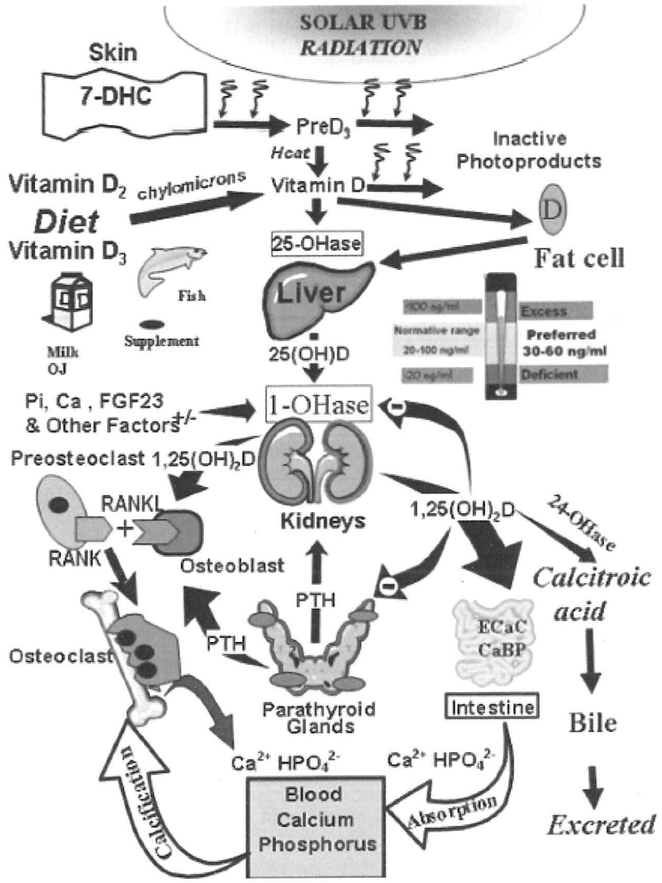
Figure 2-14 Vitamin D metabolism




During exposure to sunlight, 7-dehydrocholesterol in the skin is converted to previtamin D3. PreD3 immediately converts by a heat dependent process to vitamin D3. Excessive exposure to sunlight degrades previtamin D3 and vitamin D3 into inactive photoproducts. Vitamin D2 and vitamin D3 from dietary sources is incorporated into chylomicrons, transported by the lymphatic system into the venous circulation. Vitamin D (D represents D2 or D3) made in the skin or ingested in the diet can be stored in and then released from fat cells. Vitamin D in the circulation is bound to the vitamin D binding protein which transports it to the liver where vitamin D is converted by the vitamin D-25-hydroxylase to 25-hydroxyvitamin D [25(OH)D]. This is the major circulating form of vitamin D that is used by clinicians to measure vitamin D status. It is biologically inactive and must be converted in the kidneys by the 25-hydroxyvitamin D-1α-hydroxylase (1-OHase) to its biologically active form 1,25-dihydroxyvitamin D [1,25$(OH)_2$D]. Serum phosphorus, calcium, fibroblast growth factors (FGF-23) and other factors can either increase (+) or decrease (-) the renal production of 1,25$(OH)_2$D. 1,25$(OH)_2$D feedback regulates its own synthesis and decreases the synthesis and secretion of parathyroid hormone (PTH) in the parathyroid glands. 1,25$(OH)_2$D increases the expression of the 25-hydroxyvitamin D-24-hydroxylase (24-OHase) to catabolize 1,25$(OH)_2$D to the water soluble biologically inactive calcitroic acid which is excreted in the bile. 1,25$(OH)_2$D enhances intestinal calcium absorption in the small intestine by stimulating the expression of the epithelial calcium channel (ECaC) and the calbindin 9K (calcium binding protein; CaBP). 1,25$(OH)_2$D is recognized by its receptor in osteoblasts causing an increase in the expression of receptor activator of NFκB ligand (RANKL). Its receptor RANK on the preosteoclast binds RANKL which induces the preosteoclast to become a mature osteoclast. The mature osteoclast removes calcium and phosphorus from the bone to maintain blood calcium and phosphorus levels. Adequate calcium and phosphorus levels promote the mineralization of the skeleton.

(Holick, 2010) [Reproduced with permission] (N.B. Milk is fortified with vitamin D in the USA but not in the UK or South Africa. In the UK only margarine and spreading fats are statutorily fortified with vitamin D).



Renal 1,25$(OH)_2$D is secreted into the circulation and binds to DBP and circulates to tissues involved in calcium and phosphorus supply (e.g., bone, gut and parathyroid glands), where it acts as an endocrine modulator. In contrast to 25(OH)D, 1,25$(OH)_2$D is present in picomolar concentrations in the circulation and has a very short $t_{½}$, of 4 – 6 hours. 1,25$(OH)_2$D produced in extra renal tissues (e.g., macrophages) acts in a local autocrine or paracrine fashion to exert its effect and does not appear in the systemic circulation except in rare circumstances, such as sarcoidosis, in which CYP27B1 in activated macrophages is capable of generating enough 1,25$(OH)_2$D to be measurable in the blood (Adams *et al*, 2012). The function of 1,25$(OH)_2$D in these target tissues is to prompt genomic responses via interactions with the vitamin D receptor (VDR). The VDR, upon binding to 1,25$(OH)_2$D, heterodimerizes with other nuclear hormone receptors, in particular the family of retinoid X receptors (RXR). This VDR-RXR complex then binds to special DNA sequences called vitamin D response elements (VDRE) in the promoters of genes which it regulates. This is in contrast to vitamin D or 25(OH)D which do not readily bind to VDRs (Prentice *et al*, 2008; Dowd *et al*, 2010).

PTH concentration is an important marker of vitamin D and calcium metabolism. An elevated PTH is a risk factor for osteoporosis in the elderly (Prentice *et al*, 2008) and supplemental vitamin D can lower it (Malabanan *et al*, 1998). PTH is also found to be raised in HIV-positive patients receiving the ARV drug, tenofovir (Masiá *et al*, 2012), however it is not clear if this is the result of increased production or decreased excretion. It may be that impaired renal function associated with HIV infection disrupts the normal PTH axis. The secretion of PTH into the bloodstream is stimulated by a decrease in plasma ionised calcium (iCa), and PTH acts to regularise iCa which, in health, is maintained within a very narrow range. A decrease in iCa, below this narrow physiological range, may be the result of low dietary calcium intake, poor calcium absorption or low vitamin D. High dietary phosphorus intakes or high serum phosphate concentrations (such as in renal failure) may induce PTH secretion via effects on FGF23 and 1,25$(OH)_2$D (see Section 2.3.8.)

The inverse relationship between PTH and 25(OH)D has suggested its use as a proxy for vitamin D status. However, the non-specificity of PTH, the lack of consistency between studies, and the inter- and intra-individual variability limits its use as a biomarker of vitamin D status. Furthermore, since most studies of PTH in this context have been in Caucasian populations it is not possible to define adequately its role, if any, as a proxy vitamin D status marker in non-Caucasian populations. However, studies from China and The Gambia (Yan *et al*, 2003; Aspray *et al*, 2005) have demonstrated elevated PTH concentrations in populations with low-moderate calcium intakes, and that the inverse relationship with aBMD and fracture found in Caucasians is not replicated. Therefore, the relationship between PTH, vitamin D status, and calcium intake in different population groups needs to be better understood (Prentice *et al*, 2008).

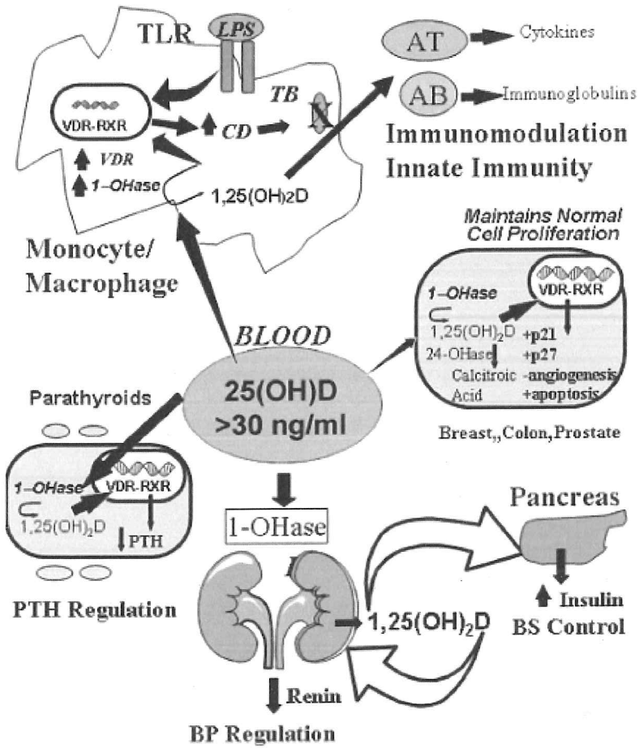
There is a negative relationship between vitamin D status and adiposity, with some speculation that vitamin D and its metabolites may be permanently sequestered in adipose tissue and therefore unavailable (Wortsman *et al*, 2000; Sulistyoningrum *et al*, 2012). Others regard the negative association to be a volumetric dilution effect rather than a manifestation of vitamin D sequestration into adipose tissue per se (Drincic *et al*, 2012). There is some evidence that vitamin D mobilises following rapid weight loss, such as post-bariatric surgery, suggesting the liberation of vitamin D from adipose tissue (Lin *et al*, 2011). There is a debate about whether this mobilisation of vitamin D has potential effects on individuals who experience rapid changes in adiposity.

VDR are present in many different cell types including those of the breast, muscle, and immune cells such as macrophages. Because of the widespread distribution of VDR, $1,25(OH)_2D$ acts, or has the potential to act, on a wide range of target tissues and organs. Therefore, vitamin D supply has the potential to affect the body systems other than musculoskeletal (Figure 2-15) and has been increasingly recognised for potential effects on non-skeletal health.



There are questions about the optimal ways of ensuring appropriate vitamin D status in populations. Sunlight has both UVA and UVB components (UVC is mostly filtered out by the Earth's ozone layer); only the UVB wavelengths can generate the reactions for endogenous vitamin D synthesis (Section 2.3.1). However, both act as drivers of dermal carcinogenesis via inducing DNA-damage (Sage *et al*, 2012) and there are concerns about advocating sunshine exposure as a means to ensure population vitamin D adequacy. It has been argued that the global burden of disease caused by excess ultraviolet exposure, most notably skin cancer, is far outweighed by diseases associated with poor vitamin D status (via low UV exposure) (Lucas *et al*, 2008). In terms of estimated disability-adjusted life years (DALY) this equates to a loss of 1.6 million with respect to over-exposure to UV compared to 3.3 billion with under-exposure (Lucas *et al*, 2008).

**Figure 2-15 Non-skeletal functions of vitamin D**




When a monocyte/macrophage is stimulated through its toll-like receptor 2/1 (TLR2/1) by an infective agent such *Mycobacterium tuberculosis* (TB), or its lipopolysaccharide (LPS) the signal upregulates the expression of vitamin D recep (VDR) and the 25-hydroxyvitamin D-1-hydroxylase (1-OHase). 25(OH)D levels > 30 ng/ml provides adequate substrate for the OHase to convert it to 1,25(OH)2D. 1,25(OH)2D returns to the nucleus where it increases the expression of cathelicidin (C which is a peptide capable of promoting innate immunity and inducing the destruction of infective agents such as TB. It is a likely that the 1,25(OH)2D produced in the monocytes/macrophage is released to act locally on activated T (AT) and activate (AB) lymphocytes which regulate cytokine and immunoglobulin synthesis respectively. When 25(OH)D levels are ≈30 ng/m reduces risk of many common cancers. It is believed that the local production of 1,25(OH)2D in the breast, colon, prostate, other cells regulates a variety of genes that control proliferation including p21 and p27 as well as genes that inhibit angiogen and induced apoptosis. Once 1,25(OH)2D completes the task of maintaining normal cellular proliferation and differentiation induces the 25-hydroxyvitamin D-24-hydroxylase (24-OHase). The 24-OHase enhances the metabolism of 1,25(OH)2D calcitroic acid which is biologically inert. Thus, the local production of 1,25(OH)2D does not enter the circulation and has influence on calcium metabolism. The parathyroid glands have 1-OHase activity and the local production of 1,25(OH)2D inhi the expression and synthesis of PTH. The production of 1,25(OH)2D in the kidney enters the circulation and is able to d regulate renin production in the kidney and to stimulate insulin secretion in the β-islet cells of the pancreas

(Holick, 2010) [Reproduced with permission] (N.B. there are many other putative effects of vitamin D such as in multiple sclerosis).



### 2.3.5 Vitamin D, the bone and muscle unit

As described above, vitamin D is essential for good bone health. An adequate supply and the body's ability to appropriately utilise available vitamin D is key to preventing rickets in children and osteomalacia in children and adults. Vitamin D is also essential for the prevention of osteoporosis in older adults, and also plays a key role in muscle function, potentially decreasing the rates of falls and fragility fractures. Skeletal muscle integrity is required to facilitate posture and ambulation, a disruption in this integrity can increase the risk of falling. Skeletal muscle possesses VDR and may require vitamin D for maximum function via myocyte calcium handling and muscle cell proliferation (Ceglia *et al*, 2013). A metanalysis of 5 trials comprising 1237 older subjects showed that increased vitamin D intake reduced falls by 22% when compared with calcium alone or placebo (Bischoff-Ferrari *et al*, 2006; Holick, 2007). In contrast, a 2012 Cochrane review found that in older people living in the community vitamin D supplementation did not reduce the "rate of falls (RR 1.00, 95% CI 0.90 to 1.11; seven trials; 9324 participants) or risk of falling (RR 0.96, 95% CI 0.89 to 1.03; 13 trials; 26,747 participants), but may do so in people with lower vitamin D levels before treatment" (Gillespie *et al*, 2012). The authors state that lower blood 25(OH)D concentrations, rather than lower intakes, prior to vitamin D supplementation was most likely to increase falls rate and risk.

### 2.3.6 Vitamin D and fracture

Indirectly, aBMD is a strong predictor of fragility fracture risk in older people from countries at high risk, and possibly in adolescents although this is not proven. Several RCTs have demonstrated the beneficial effects of vitamin D supplementation on aBMD; therefore it might be assumed that good vitamin D status will decrease fracture risk via effects on aBMD and possibly on suppression of PTH (Bischoff-Ferrari *et al*, 2006), and muscle function. However the evidence is mixed.

Data from the UK Randomised Evaluation of Calcium OR vitamin D (RECORD) study did not demonstrate an anti-fracture effect (with supplements of 800 IU/day of cholecalciferol) (Grant *et al*, 2005). Similarly, a study by Porthouse *et al* did not report a

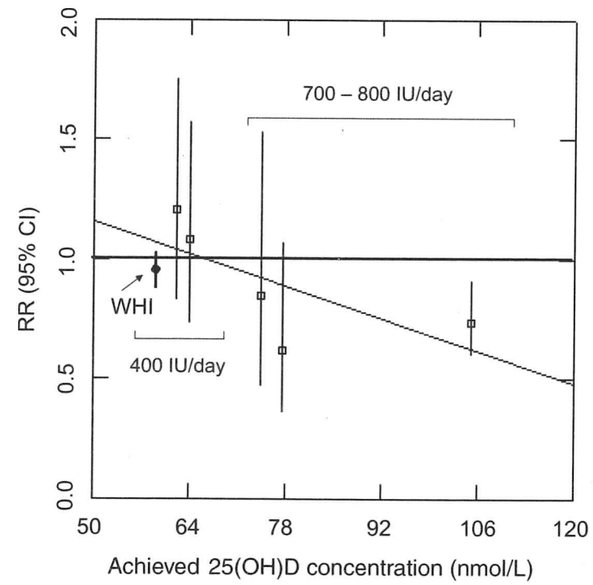
decrease in fracture risk with 800 IU/d of vitamin D plus 1000 mg calcium/d in women at high risk (Porthouse *et al*, 2005).

In addition, the DIPART (Vitamin D Individual Patient Analysis of Randomized Trials) Group in a pooled analysis of 68,500 individuals demonstrated that "10-20 mcg (of vitamin D) is not effective in preventing fractures. By contrast, calcium and vitamin D given together reduce hip fractures and total fractures, and probably vertebral fractures, irrespective of age, sex, or previous fractures" (Abrahamsen, 2010; DIPART Group, 2010). Other studies support the role of supplemental vitamin D with calcium but not vitamin D alone in reducing fracture risk (Ott, 2012a). Therefore the role of vitamin D supplementation in the prevention of fracture is controversial, although it is plausible that good vitamin D status has a beneficial effect on aBMD and therefore an indirect effect on reducing fracture risk.

Bischoff-Ferrari *et al* have attempted to determine the 'antifracture efficacy' of oral vitamin D supplementation (Bischoff-Ferrari *et al*, 2006). In a meta-analysis of 5 RCTs looking at hip fracture rates (n=9294) and 7 RCTs of non-vertebral fracture, vitamin D intakes of 700-800 IU/day (17.5-20 mg/day) decreased the relative risk (RR) of hip fracture by 26% and non-vertebral fracture by 23% when compared with calcium or placebo. On the basis of these findings the authors suggested that "optimal fracture prevention occurred in trials with mean achieved 25(OH)D ≈100 nmol/l" by high dose supplementation i.e., 700-800 IU/d (Bischoff-Ferrari *et al*, 2006) which is at variance with the IOM's recommendation (see Section 2.3.1). Figure 2-16 illustrates the relative risk (RR) of hip and non-vertebral fracture at different serum concentrations of 25(OH)D. The top graph is the RR for hip and the bottom for non-vertebral fracture. These show that the Women's Health Initiative (WHI) and RECORD studies had RR values with CIs that crossed one, and that those studies with an RR demonstrating a protective effect were supplementing with 700-800 IU/day, which equates to serum 25(OH)D concentrations of approximately 70—110 nmol/l.



**Figure 2-16 Relative risks of hip and non-vertebral fracture**




Relative risks (RRs: □) of hip fracture (top) and non-vertebral fracture (bottom) between subjects who took vitamin D and control subjects
Error bars represent 95% CIs. The trend line is based on series of effect sizes (□).

(Bischoff-Ferrari *et al*, 2006) [Reproduced with permission]

In a 2012 pooled analysis of 11 RCTs including 31,022 persons (mean age, 76 years; 91% women) with 1111 incident hip fractures and 3770 non-vertebral fractures, Bischoff-Ferrari *et al* described a non-significant, 10% decrease in hip and 7% decrease in the risk of non-vertebral fracture in those randomised to receive vitamin D (+/- calcium). When actual intake of vitamin D was analysed, reduction in fracture risk was seen in those with the highest intakes of vitamin D (median 800 IU daily). This was associated with a 30% reduction in the risk of hip fracture and a 14% reduction in the risk of any non-vertebral fracture. The authors concluded that, "high-dose vitamin D supplementation (≥800 IU daily) was somewhat favourable in the prevention of hip fracture and any nonvertebral fracture in persons 65 years of age or older" (Bischoff-Ferrari *et al*, 2012). Methodological flaws in this analysis have been suggested, including the fact that sunlight exposure, hence endogenous vitamin D synthesis, was not determined thereby weakening assertions made about the utility of vitamin D supplementation for reducing fracture risk (Ott, 2012b).

There are currently no specific data regarding vitamin D status and fracture end point in HIV-positive individuals. In the largest population-based study of HIV-associated fracture, Triant *et al* do not present data on the vitamin D status of the fracture cases or controls (Triant *et al*, 2008). In her review of HIV-associated bone fracture in recent cohort studies, Hoy comments that vitamin D deficiency is common in the cohorts examined, but that the prevalence was no different to the general population, and no association with aBMD was noted in cross-sectional studies. She also commented that cross-sectional data from the 'Study to Understand the Natural History of HIV and AIDS in the Era of Effective Therapy' (SUN) cohort matched with NHANES data revealed within the HIV-positive population a "high prevalence (70%) of vitamin D insufficiency and deficiency which was comparable with the general population (79%)", the definition of deficiency was based on the SUN study and was set at 75nmol/l. "However, there was no association between aBMD and vitamin D insufficiency or deficiency" (Dao *et al*, 2011; Hoy, 2011). These findings have been replicated in a cross-sectional study of HIV-



positive and negative Hispanic and African-American postmenopausal women (Rodriguez *et al*, 2009; Stein *et al*, 2011). However, none of these studies had fracture endpoint data in HIV-positive patients.

### 2.3.7 Vitamin D and non-skeletal health

A detailed analysis of the evidence for the non-skeletal role of vitamin D is beyond the scope of this thesis; for a summary see Holick (2007) and Martineau *et al* (2007a). These reviews detail the evidence of the relationship between vitamin D status and non-skeletal outcomes from in vitro, preclinical, animal and epidemiological studies. Non-skeletal outcomes examined include malignant, infectious, autoimmune, endocrine, and psychiatric conditions.

In recent years, a burgeoning body of research has examined the effect of vitamin D on immune function and its importance in conditions as diverse as tuberculosis and psoriasis (Wilkinson *et al*, 2000; Mathieu *et al*, 2002; Cantorna *et al*, 2004; Holick, 2006; Stoffels *et al*, 2006; Holick, 2007; Martineau *et al*, 2007a; Martineau *et al*, 2007b; Adams *et al*, 2008; Alagarasu *et al*, 2009) as well as neoplastic diseases (Holick, 2007; Prentice *et al*, 2008). These data suggest that vitamin D may have a particular role in protection from infectious disease (Coussens *et al*, 2012; Khoo *et al*, 2012).

$1,25(OH)_2D$ has paradoxical effects on the immune system suggesting that it is what Cantorna calls a "selective regulator" of immune function (Cantorna *et al*, 2004). $1,25(OH)_2D$ can be seen either as immune-suppressive or immune-stimulating depending on the type of immune response elicited (infectious vs. autoimmune). This is almost certainly a consequence of altered transportation and conversion of 25(OH)D as well as differences in VDR and intracellular effects of $1,25(OH)_2D$. It is not clear how these effects of $1,25(OH)_2D$ relate to vitamin D status, as measured by 25(OH)D, and it is conceivable that the immune-modulating mechanisms are different. The role of T-helper (Th) cells is key to antigen-specific immune responses, it is also these cells that are the major target for HIV infection (see Section 2.1.1). Th1 cells secrete the inflammatory cytokines interleukin 2 (IL2), interferon gamma (IFNγ) and tumour

necrosis factor alpha (TNFα). Such Th1 responses are required for strong cell-mediated immunity (Cantorna *et al*, 2004). In contrast, Th2 lymphocytes secrete IL4 and IL5; these are key to humoral (antibody) mediated responses in concert with B-lymphocytes. Both Th1 and Th2 lymphocytes are targets for $1,25(OH)_2D$ so express VDR; $CD_4$ positive T-lymphocytes also express VDR. Further work on innate immunity suggested that vitamin D and VDR play a role in human defence against pathogens. "In innate immune responses, activation of Toll-like receptors (TLRs) triggers direct antimicrobial activity against intracellular bacteria … We report here that TLR activation of human macrophages up-regulated expression of the vitamin D receptor and the vitamin D-1-hydroxylase genes, leading to induction of the antimicrobial peptide cathelicidin and killing of intracellular Mycobacterium tuberculosis" (Liu *et al*, 2006). Holick has stated, "when serum levels of 25-hydroxyvitamin D fall below 20 ng per millilitre (50 nmol per liter), the monocyte or macrophage is prevented from initiating this innate immune response" (Holick, 2007). This may have direct implications in terms of control of other intracellular pathogens such as HIV, and indirect effects, e.g. on TB since it is a major cause of pathology in HIV-infected patients.

Patients with, or at risk of, diseases such as HIV infection, may require 25(OH)D concentrations above that needed to maintain bone health. HIV-positive individuals may have higher demands for vitamin D to meet the increased immune cell turnover, and as a result of co-morbidities such as TB. Those with lower vitamin D status may have decreased mucosal immunity, so are at increased risk of infection. However, such hypotheses have not been proven; controlled studies with disease end points are required. It remains to be seen if the associations observed between vitamin D status and non-skeletal disease are causal, or indeed if poor status predisposes to infectious disease or if good status is protective (Ross, 2010).

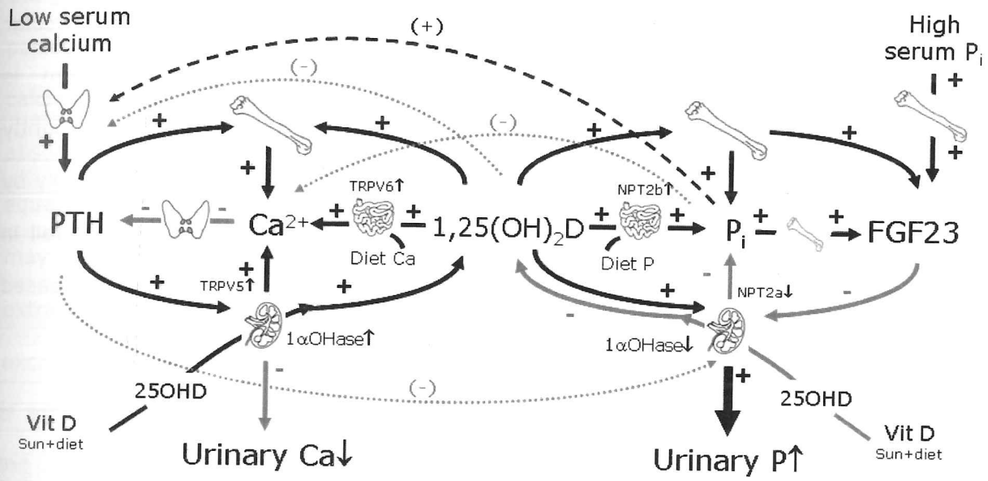
### 2.3.8 Regulation of calcium and phosphate homeostasis

Vitamin D has an essential role in maintaining calcium and phosphate homeostasis (Figure 2-17). Calcium and phosphate metabolism is under classical hormonal feedback



control. Each element of the system interacts to maintain optimal extracellular calcium and inorganic phosphate concentrations. Bone provides an abundant supply of these ions and also acts as a buffer against an excessive supply of these minerals entering the extracellular fluid from dietary sources.

**Figure 2-17 A schematic of the calcium-phosphate-vitamin D homeostatic system**




(Prentice *et al*, 2008) [Reproduced with permission]

The renal system is central to the control of calcium and phosphate homeostasis through the excretion of calcium and phosphate in the urine and the conversion of 25(OH)D to $1,25(OH)_2D$ (under action of PTH), which is responsible for regulating calcium absorption in the small intestine. The kidneys have the capacity to filter 20% of cardiac output per minute and produce in the region of 180L of filtrate each day, 99% of which is reabsorbed, resulting in approximately 1.4L of urine excreted daily. This urine contains metabolic waste as well as appropriate calcium and phosphate to maintain extracellular fluid balance of these ions. During times of fast, the kidneys reabsorb 99% of filtered calcium and 95% of phosphate ions. Most of this reabsorption occurs at the proximal convoluted renal tubule; at this site calcium reabsorption is coupled with sodium reabsorption. Postprandially this reabsorptive capacity falls dramatically and as a result

there is renal loss of these ions in order to maintain physiological concentrations in the extracellular fluid (Anderson *et al*, 2012).

#### 2.3.8.1 Calcium

Calcium is the most abundant mineral in the body. A 70 kg adult contains approximately 1.2 kg of calcium. It is estimated that 99% of the body's calcium is present in bone in the form of hydroxyapatite. The remaining 1% is distributed between body fluids and tissue. Calcium has numerous physiological functions; key ones include neuromuscular conduction and regulation of skeletal and cardiac muscle. Serum calcium is approximately 40% albumin-bound and the concentration of the ionised form is tightly regulated. This keeps total calcium within 2.02-2.65 mmol/l. It is regulated primarily by PTH and $1,25(OH)_2D$; increases in which result in a rise in serum calcium (and fall in phosphate) due to increased calcium and phosphate reabsorption from bone, decreased renal calcium excretion, and increased phosphate excretion from the renal tubule.

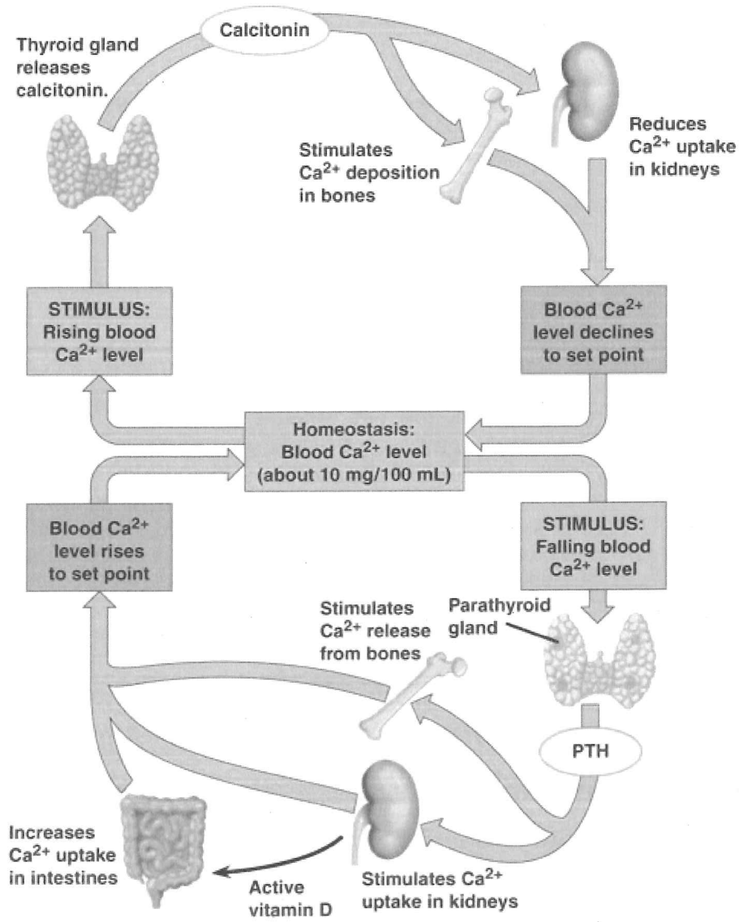
The diet is the only source of calcium; under low calcium intake conditions there is increased intestinal absorption to maintain calcium balance. Rich dietary sources are dairy products and fortified cereals with smaller contributions by seeds, beans, and green leafy vegetables. Calcium intakes vary widely between populations and at different stages of the lifecycle, and absorption of ingested calcium differs depending on prevailing physiological requirements and dietary factors. There is obligatory loss of calcium in sweat, urine, and faeces. The adaptive response to low calcium intake is increased conversion of 25(OH)D to $1,25(OH)_2D$ in the kidney via the actions of PTH. PTH responds to low iCa via calcium sensing receptors that are located in the cell membranes of the parathyroid gland. Under low calcium intake conditions, there are slight decreases in iCa in the extracellular fluid, which stimulates PTH production and increased synthesis of $1,25(OH)_2D$ (via 1-alpha-hydroxylase, CYP27B1) in the proximal renal tubule. Calcium uptake from the gut is by active absorption mediated by $1,25(OH)_2D$. In times of high calcium intakes there is non-regulated paracellular diffusion of calcium from the gut, between enterocytes. The renal threshold for calcium is in the region of 4mg/kg lean



mass/day. If calcium intake exceeds approximately 2300mg/day, renal thresholds may be exceeded with resultant soft tissue calcification (Klemmer *et al*, 2012).

Calcium homeostasis is regulated by a complex interaction between the kidneys, gut, parathyroid glands, and skeleton (see Figure 2-18). As well as regulating gut absorption of calcium (via production of $1,25(OH)_2D$) the kidneys also reabsorb calcium present in the glomerular filtrate via tubular reabsorption. They filter in the region of 10g calcium per day, only 200 or 120 (men and women) mg of which passes into the urine. Urinary calcium concentrations are maintained across a wide range by modulation of gut calcium absorption, so that in young adults the net amount absorbed in the small intestine equals that excreted in urine. Even small decreases in glomerular filtration rate (GFR) may result in decreased urine calcium excretion (Klemmer *et al*, 2012). Expansion of the extracellular space by increased sodium intake increases obligatory urinary calcium excretion, which facilitates urinary excretion of sodium ions. However, it not established if high sodium intakes results in net bone loss (Blackwood *et al*, 2001). The tight regulation of serum calcium concentration is facilitated by the effect of PTH on bone. The calcium in hydroxyapatite is not able to buffer minute-by-minute changes in extracellular calcium concentration; the calcium present in the fluid compartment of the bone serves this purpose. Increased concentrations of PTH promote the release of ionic calcium from the bone envelope. In contrast, after calcium ingestion, the skeleton buffers the rise in iCa from extracellular fluid by removing it from the circulation (Klemmer *et al*, 2012).

**Figure 2-18 Calcium homeostasis**




(University of Birmingham at Alabama) [Reproduced under auspices of

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### 2.3.8.2 Phosphate

Phosphate homeostasis differs from that of calcium because it is mostly regulated at the site of the proximal renal tubule rather than at the small intestine. Dietary phosphorus is readily available especially in Western diets where phosphorus is added to processed foods in the form of buffers. Rich food sources are milk, grains, and protein-rich foods.

Of the 1 kg of phosphorus contained in the body, approximately 80% is in the form of the calcium phosphate salts comprising the inorganic component of bone. The remainder

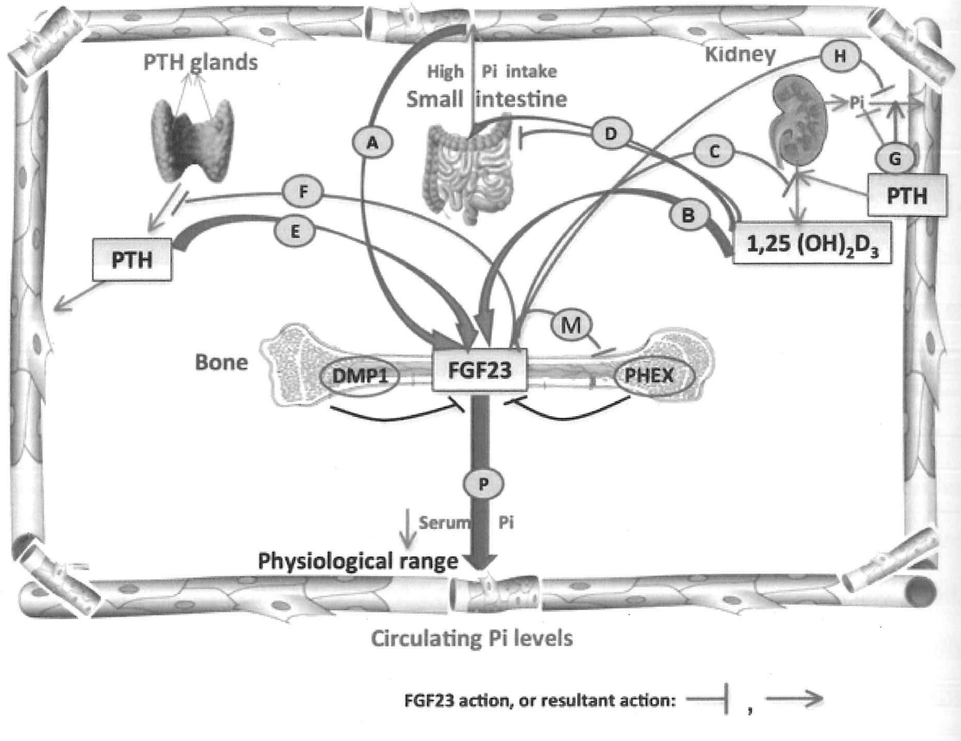


is distributed throughout the cells of the body, mainly in the form of organic phosphorus forming part of phospholipids and phosphoproteins. In serum the majority of inorganic phosphorus exists in free form (phosphate) with approximately 15% protein bound.

Measurements of inorganic phosphate in serum and urine are used in the diagnosis of various conditions such as parathyroid disease and renal disease. In the context of HIV-disease, serum phosphate is an important analyte; it has been associated with exposure to tenofovir which can result in renal phosphate wasting and subsequent effects on bone health.

A typical phosphorus intake of 1400mg/day results in 900mg renal and 500mg stool excretion. As intake and output are almost equal, healthy adults are in zero phosphate balance. Between 60-70% of phosphorus uptake takes place in the small intestine via the sodium/phosphate transporter. Phosphate homeostasis is largely independent of vitamin D but depends on actions of PTH and Fibroblast Growth Factor 23 (FGF-23). FGF-23 is a phosphatonin hormone produced by osteocytes that promotes urine phosphate excretion. FGF-23 also reduces the activity of the renal 1-alpha-hydroxylase and hence the production of $1,25(OH)_2D$. Following a phosphate load, PTH and FGF-23 concentrations increase in extracellular fluid resulting in a reduction in proximal tubular reabsorption of the ion. This has the net result of increasing urinary phosphate excretion. The osteocyte-related, noncollagenous matrix protein Dentin Matrix Protein1 (DMP1) and phosphate-regulating gene with homology to endopeptidases on the X chromosome (PHEX) regulate phosphate metabolism through degradation of FGF-23 (Martin *et al*, 2011).

**Figure 2-19 Phosphate homeostasis**




Negative regulation of phosphate homeostasis by FGF23. FGF23 is negatively regulated by bone signalling mechanisms (DMP1 and PHEX), and is positively regulated by systemic factors (serum Pi, $1,25(OH)_2D_3$, PTH). A-H: intestine–bone–kidney–parathyroid gland feedback loops. (A) High absorbed dietary Pi stimulates FGF23 secretion. (B) $1,25(OH)_2D_3$ also stimulates FGF23 secretion. (C) In turn, FGF23 suppresses kidney production of $1,25(OH)_2D_3$, resulting in (D) decreased potential $1,25(OH)_2D_3$-dependent Pi absorption by the intestine. (E) PTH also up-regulates FGF23 synthesis and secretion, and in turn (F) PTH synthesis is decreased by FGF23. (G) As a result, potential PTH-dependent inhibition of renal Pi reabsorption is decreased. (H) Overall elevated levels of FGF23 result in inhibited renal Pi reabsorption (phosphaturic action). (M) Local bone FGF23 excess results in inhibited mineralization. (P) The sum consequence of FGF23 action is to decrease excess serum Pi to a physiological range.

(Sapir-Koren *et al*, 2011) [Reproduced with permission]

Ionic phosphate concentration, in extracellular fluid, is less tightly regulated than calcium and ranges in adults between 0.83 – 1.5 mmol/l in the fasting state (Anderson *et al*, 2012). Other factors such as elevated concentrations of insulin, glucose and catecholamines, and rapid bone mineralisation also play a role in the redistribution of



phosphate between extracellular and bone or cell components, resulting in decreased plasma concentrations (Drezner, 2008).

## 2.4 Effects of HIV infection, ART, and associated conditions on bone health

As discussed at the beginning of this thesis (Section 1.1), there is a growing clinical perception of, and experience with, HIV-associated bone disease. Many clinical and observational studies correlate HIV infection with bone loss. This section will explore these issues and, where possible, the mechanisms underpinning them and isolating, where possible, effects of HIV infection, ART exposure and non-direct, HIV-related factors on bone health, although overlap is inevitable so sometimes these factors will be considered together.

### 2.4.1 HIV and bone health

The Gilead 903 study (Powderly *et al*, 2005) has provided important information on pre-ART bone data in HIV-infected individuals. In 600 HIV-positive, ART-naïve individuals, the baseline rates of DXA-defined osteopaenia at the lumbar spine (LS) were 23% and 28% in each pre-treatment arm (tenofovir vs. stavudine). Respective LS osteoporosis rates at baseline were 3% and 4% respectively. The lower LS T-scores correlated with male sex and the 'traditional' risk factors of lower weight and advancing age (Bruera *et al*, 2003; Brown *et al*, 2006a). These baseline low aBMD data suggest HIV infection may itself contribute to bone loss because low aBMD pre-dated ART exposure; however this study lacked a control group.

Low (Z-score <-2.0) aBMD has been observed in 46% of patients with untreated HIV infection and high HIV RNA concentrations in plasma (Fausto *et al*, 2006), and was also associated with HIV infection of a longer duration compared to those with more recent infection (Mondy *et al*, 2003). Conversely, patients with low HIV RNA and high $CD_4$ counts have higher aBMD, compared to those with lower $CD_4$ counts (Dolan *et al*, 2006), although one confounding factor, in such observations, is the co-exposure of such

patients to ART. In another study, low level HIV- viraemia was associated with a greater risk of osteoporosis using WHO criteria (Cazanave *et al*, 2008).

An influential meta-analysis of 20 trials by Brown and Qaqish in 2006 demonstrated that 67% of the HIV-positive individuals (n=884) studied had low aBMD; 15% of these had osteoporosis. These pooled data gave odds ratios of 6.4 and 3.7 of low aBMD and osteoporosis respectively compared with HIV-negative controls (n=654). The main methodological problem with all these studies, and doubtless accounting for many of the discrepancies in the data, is that they are all observational and therefore causality cannot be assigned (Post *et al*, 2011).

It has not been conclusively demonstrated in well-designed studies that low aBMD predicts fracture risk in HIV-positives although evidence is emerging to support this proposition (Collin *et al*, 2009; Grund *et al*, 2009). Cross-sectional studies have reported increased vertebral fractures in HIV-positive compared to HIV-negative controls (26.9% and 12.9% $p$=0.002) (Torti *et al*, 2012) but causality cannot be inferred from these studies.

In the context of HIV, a study by Arnsten *et al*, in older men (mean age 55 years) noted HIV infection was independently associated with modestly reduced aBMD in ageing men, and decreased aBMD was associated with increased fracture risk but did not see an association between ART use and aBMD loss (Arnsten *et al*, 2007). Moreover, in this study the negative relationship between HIV and aBMD was only present in overweight or obese individuals. This suggests that a higher BMI, also associated with slower HIV progression, may lessen the effect of HIV on aBMD (ibid.). This raises the important issue of the role of body weight in skeletal and non-skeletal disease in HIV-infected patients. A 2010 study from Johannesburg has shown that obesity was associated with less TB-associated mortality in a HIV-positive population (Hanrahan *et al*, 2010; Hurley *et al*, 2011), these data challenge notions of 'healthy' BMI applied to African HIV-positive individuals and warrant further exploration.



#### 2.4.1.1 Age, gender, and lifestyle

HIV-positive individuals, in the West at least, have higher rates of traditional risk factors for osteoporosis, e.g. smoking and lower plasma 25(OH)D concentrations (Carr *et al*, 2001; Mondy *et al*, 2003; Dolan *et al*, 2006; Prior *et al*, 2007). Advanced HIV disease is associated with immobility (Terzian *et al*, 2009), malnutrition (Jacobson *et al*, 2003), and altered gut function (Martineau *et al*, 2007a; Wejse *et al*, 2007; MacPherson *et al*, 2009). These factors may be associated with reduced aBMD through negative effects on bone loading and nutrient intake and/or absorption.

Comorbid conditions, associated with HIV, often have shared predisposing factors with poor bone health, such as the use of medications that may influence bone directly or indirectly (via effects on calcium or vitamin D metabolism).

Some authors have reported higher prevalence of low aBMD in HIV-positive men than women (Fernandez-Rivera *et al*, 2003). The mechanisms underpinning this observation are not clear but may relate to gonadal dysfunction and low testosterone production, as measured by free testosterone (Dube *et al*, 2007). Despite the cited increased risk of low aBMD in HIV-positive men, women with HIV infection are twice as likely to have low aBMD compared to their HIV-negative peers (Brown *et al*, 2006a).

As the global HIV-positive cohort ages (Yin *et al*, 2009) age and gender will be key determinants of bone loss in these patients. However, few studies have specifically addressed the issue of aBMD in elderly HIV patients. Jones *et al* compared 57 HIV-positive with 47 HIV-negative subjects aged over 55 years in a cross sectional study. The mean age was 61 years in HIV cases and 62 in HIV-negative controls (Jones *et al*, 2008). This study found that aBMD at the hip was significantly lower in HIV-infected patients versus controls, and that tenofovir use was particularly associated with low spine aBMD (PI use was associated with low hip aBMD) and the authors speculated that the loss of trabecular bone in the lumbar spine and cortical bone of the hip represent different mechanisms by which PIs and tenofovir affect bone turnover.

Differences in leptin and adiponectin concentrations, and their relationships with adiposity and bone, in the context of HIV infection and/or ART exposure, may also be involved and require exploration although published studies suggest that there is an inverse relationship between leptin and bone mass in HIV-positive patients treated with ART (Madeddu *et al*, 2009). This may be mediated by disturbances in fat distribution and integrity seen in association with HIV-associated lipodystrophy (Rao *et al*, 2012)

Injection drug use (IDU) (Madeddu *et al*, 2004) and associated methadone replacement in men and women is also associated with reduced aBMD (Arnsten *et al*, 2006; Arnsten *et al*, 2007), however, these associations may be primarily related to low BMI. Smoking and alcohol excess, and decreased mobility have been associated with low aBMD in HIV-infected patients as well as in the general population (Mondy *et al*, 2003; Dolan *et al*, 2006; Collin *et al*, 2009; Lo Re 3rd *et al*, 2009). These findings are not, however, replicated in all studies (Prior *et al*, 2007). IDU is anecdotally reported to be low in Southern Africa but firm data are lacking. Nevertheless, recreational, non-IDU drug use is thought to be increasing generally and may disproportionately affect women (Parry *et al*, 2009). There are anecdotal reports of smoking crushed-up ARV tablets in South Africa. This so called 'Whoonga' is used as a recreational drug to promote hallucinations (Fihlani, 2011).

#### 2.4.1.2 Pathophysiological mechanisms

The normal process of bone remodelling is disrupted in HIV infection by uncoupling bone formation and resorption, indicated by increased markers of bone resorption and stable markers of formation over time (Aukrust *et al*, 1999). The pathophysiological mechanisms of HIV-associated increases in bone turnover, and related histomorphological changes of HIV and/or ART-associated bone loss, and effects on aBMD have yet to be fully elucidated. The relationship between HIV infection and osteoporosis is multifactorial. Some authors have speculated that specific components of the HIV coat and viral proteins (such as gp120, (see Figure 2-2) or p55 gag (Walker-Bone, 2012)) may increase osteoclast activity (Brown *et al*, 2006a). This direct effect



may explain why duration of HIV infection appears to be a better predictor of low aBMD (Bruera *et al*, 2003) than specific ART regimes (Seminari *et al*, 2005). HIV has direct apoptotic effects on osteoblasts via up-regulation of TNFα (Gibellini *et al*, 2008). Other studies suggest that certain PIs induce bone loss by preventing inhibition of RANKL (Fakruddin *et al*, 2003). However, the underlying effects of HIV and/or ART exposure on the bone remodelling unit requires clarification as it may provide insights into how to abrogate the associated bone loss. It is also worth considering that the cells of the immune system, directly influenced by HIV (namely T and B lymphocytes), are involved in the action of oestrogen on the skeleton (Raisz *et al*, 2008). This may suggest a potential common mechanism underpinning HIV-associated bone loss.

Inflammatory conditions such as Immune Reconstitution Inflammatory Syndrome (IRIS), associated with rapid immune recovery after introduction of ART, may require a prolonged course of high-dose corticosteroids and bed rest, both of which result in bone loss. Brown has suggested that low aBMD is associated with other HIV-associated metabolic abnormalities such as lipodystrophy and glucose intolerance (Brown *et al*, 2004; Brown *et al*, 2006a). However, it is difficult to establish any causal link between these associations, and they may have been the result of ART use or chronic HIV infection and associated cytokine dysregulation leading to greater tissue TNFα concentrations, or to disturbances of acid-base homeostasis. A reduction in extracellular pH, from whatever source, directly enhances osteoclastic activity and increases resorption pit formation. The alkaline skeleton acts as a buffer to maintain acid-base homeostasis and has parallels with the dietary 'ash' hypothesis which postulates that chronic, low-level acidosis results in increased bone resorption and urinary calcium excretion (New, 2002; New *et al*, 2004; Fenton *et al*, 2011).

#### 2.4.1.3 Osteoporosis diagnosis and treatment in HIV

Algorithms for investigation and treatment of low aBMD to prevent fracture in HIV-infected subjects are required to fill "one of the most important knowledge gaps in the field" (Brown *et al*, 2006a). However at present, the International AIDS Society USA

does not support routine screening for low aBMD (Schambelan *et al*, 2002). The UK and South Africa do not have screening programmes. Furthermore, as yet it is unclear if HIV infection is considered a sufficient additional risk factor to warrant screening for low aBMD in premenopausal women. Anecdotally, some UK clinicians use HIV infection instead of rheumatoid arthritis, to predict need for treatment or DXA, in the FRAX questionnaire (http://www.shef.ac.uk/FRAX/), however this strategy is not validated.

In HIV infection specific data are lacking on the best strategies to predict and prevent fracture in those with known or suspected low aBMD (Hoy, 2011; Walker-Bone, 2012). Alendronate (with calcium and vitamin D supplementation) has been shown to be safe in treating osteoporosis in HIV-positive patients (McComsey *et al*, 2007). Zoledronic acid has been demonstrated to increase aBMD for at least five years after administration in HIV positive men (Bolland *et al*, 2012a). However, the optimal duration of bisphosphonate therapy has not been established (Pollock *et al*, 2009) and it is not known if it is as efficacious at reducing fracture as in HIV-negative adults. The use of sex hormones, calcitonin and teriparatide are far less well documented in HIV-infected cases than the general population but may be considered on an individual basis (Clay *et al*, 2008).

### 2.4.2 ART and bone health

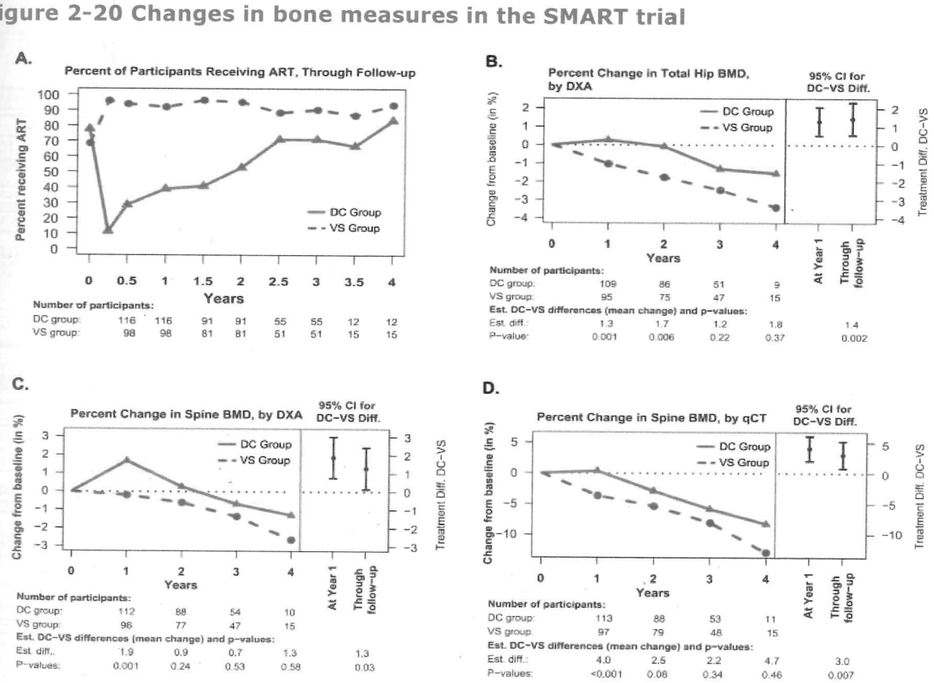
#### 2.4.2.1 Fracture

The need to manage HIV infection with long-term ART has resulted in the recognition of potential toxicities of ART exposure, and particularly, a negative effect on bone health. Current evidence shows that the prevalence of osteoporosis (Mallon, 2010; Stone *et al*, 2010; Walker-Bone, 2010; Post *et al*, 2011), and fracture (Triant *et al*, 2008) is higher in HIV positive individuals. There are case reports (Guaraldi *et al*, 2001; Forsyth *et al*, 2002) of minimal trauma fractures in patients on ART. The important Strategies for Management of ARV Therapy (SMART) study used both DXA (hip and spine) and quantitative computed tomography (qCT) (spine) to measure BMD annually. SMART was designed to compare the effects of interrupted ART treatment, guided by $CD_4$ count, and



continuous ART treatment. This was a multicentre study following patients in the USA, Australia, and Europe, the median age of participants was 44 years and 19% were female. Patients were randomised to either continuous- or interrupted-ART. In the continuous-ART arm aBMD declined more than in the intermittent group, which was stable or increased at one year. In the continuous group hip and spine aBMD declined by 0.8% and 0.4%, and vBMD by 2.4% (measured by qCT) up to one year. After 12 months, aBMD at both sites appeared to decline at similar rates to each other in the continuous-ART group (Grund *et al*, 2009). The hazard ratio for 'serious' fractures was 4.9 in the continuous-ART compared with the interrupted-ART groups. Whilst this study adjusted for factors associated with aBMD loss, such as sex, they did not define 'serious' fracture.

**Figure 2-20 Changes in bone measures in the SMART trial**




Antiretroviral therapy use and mean change in bone mineral density by treatment group. (A) percentage of participants receiving antiretroviral therapy (ART); (B) change in total hip bone mineral density (aBMD) by dual-energy radiographic absorptiometry (DXA); (C) change in spine BMD by DXA; (D) change in spine vBMD by quantitative computed tomography (qCT). Below panels B-D, estimates and P values for the DC-VS treatment differences are shown. The right-hand side of each panel shows treatment differences at year 1 and through follow-up with 95% confidence intervals (CIs). DC, drug conservation (intermittent ART group); VS, viral suppression (continuous ART group). (Grund *et al*, 2009) [Reproduced with permission]

In a French cohort study by Collin *et al* (Collin *et al*, 2009) following patients (n=1,281) who had been on ART for 10 years, the incidence of fracture was 3.3 per 1000 patient years. This may be an underestimate but is not different to the expected age-adjusted fracture rate in the general European adult population; these data suggest fracture rates were not increased in patients exposed to ART for a decade. The rate was 2.9 fold higher in those with excessive reported alcohol intake (defined as ≥5 glasses/day). Only 5/26 patients with a fracture in this study underwent bone densitometry; of these, four had osteoporosis (Collin *et al*, 2009). Other studies have reported an increase in fracture rate in HIV-positive women compared to controls despite similar aBMD (Prior *et al*, 2007). Attempts have been made to estimate the risk of HIV-associated fracture through large, retrospective hospital record reviews (Triant *et al*, 2008), in the context of drug trials (Powderly *et al*, 2005), and in cohort studies (Arnsten *et al*, 2007). These studies demonstrated increased risk of fracture in HIV-positive patients but were limited by either cross-sectional or retrospective design or lack of a control group. More recently, Yong and colleagues demonstrated that fracture risk was strongly associated with a low $CD_4$ count, although these data were in a predominantly white, male population (Yong *et al*, 2011). The use of ART or degree of HIV suppression was not associated with fracture risk.

In a 2011 publication from the 'A5224s Trial', HIV-positive patients, exposed to different ART combinations, fracture was observed in 5.6% of participants. In this RCT of treatment naïve patients, the median age was 38 years, 85% were men and 47% were white. Subjects were randomised to receive different combinations of ART (See section 2.1.2). All of these fractures (n=15) were the result of trauma. The probability of "on study" (incident) bone fractures and time to first fracture were not different across different ART-components (McComsey *et al*, 2011). Similarly in the parent 'ACTG A5202 Study', 80 participants (4.3%) reported at least one bone fracture on study. Among these, 10 (12.7%) were atraumatic. Fracture rates were balanced across the study arms, with no statistically significant differences between the NRTI ($p$=0.73) or the



NNRTI and PI components ($p$=0.57). No statistically significant difference in time to first bone fracture was seen between the NRTIs ($p$=0.71) or the NNRTI and PI components ($p$=0.49). Similarly, fracture incidence rates were similar across arms (Table 2-3).

**Table 2-3 'ACTG A5202 Study' summary**

| ART regime | Fracture rates during study (%) | Fracture incidence /100 patient-years |
|---|---|---|
| ABC/3TC & EFV | 4.7 | 1.9 |
| ABC/3tC & ATZ/r | 3.5 | 1.4 |
| TDF/FTC & EFV | 4.5 | 1.8 |
| TDF/FTC & ATZ/r | 4.5 | 1.8 |

Adapted from McComsey (McComsey *et al*, 2011).

#### 2.4.2.2 ART and aBMD

To better understand the relationship between ART use and aBMD the next section presents the literature on different classes of ART and aBMD. There are numerous reports of the effect of different ART regimes on bone and aBMD (Brown *et al*, 2009). While these studies provide important insights into HIV-related bone loss, there remains a paucity of well designed, controlled studies. Studies are divided into those that showed positive and those that demonstrate negative effects of ART exposure on bone health. Although long-term ART exposure seems to decrease aBMD, it is difficult to independently assign and quantify the effects of ART exposure in relation to those of HIV infection, and traditional risk factors for bone loss.

An important observational study by Mondy *et al* demonstrated elevated bone turnover markers during 72 week follow up in 93 HIV-infected patients on ART. In this study 85% were male and 14.7% were black, with a mean age of 41 years in those with normal aBMD. In those with low BMD 88% were male, 18% black, with a mean age of 42 years. Alterations in bone turnover markers (BTMs) appear to reverse with ARV treatment, at least initially, suggesting a temporary positive effect of ART on BTMs (Mondy *et al*, 2003). ART may simply improve bone health by decreasing the body's HIV burden, and therefore the deleterious effects of ART on bone may finally be outweighed by the benefits of limiting the effects of HIV on bone through ART.

In a study by Aukrust *et al* it was posited that ART exposure might improve markers of bone formation, with increases in osteocalcin over 24 months, compared to low osteocalcin and high CTx concentrations prior to the introduction of ART. ART exposure may cause a resynchronising of bone formation and resorption that became uncoupled (with increases in resportion) during untreated HIV infection (Aukrust *et al*, 1999).

Bolland *et al* concluded that aBMD remains relatively stable over two years despite use of ART. In fact, in their small study of 23 HIV-positive men on ART, lumbar spine aBMD increased by 2.6% from baseline compared to a 1.4% increase in 26 HIV-negative controls. aBMD at total hip and whole body remained stable in both groups. These aBMD differences occurred even though HIV-positive men were significantly more likely to smoke and had a non-significantly lower BMI than controls. While 25(OH)D and testosterone concentrations were similar at baseline, data on physical activity and drug and alcohol use were not gathered (Bolland *et al*, 2006). This study contrasts with most that describe decreasing aBMD with ongoing ART use and the authors considered BMI to be a more powerful determinant of aBMD loss than ART. However, once again, HIV and/or ART-associated differences in aBMD disappeared after correction for BMI. The authors speculated that it was weight loss associated with advancing HIV infection that was responsible for decreasing aBMD, and which is corrected by the weight gain associated with ART (Fernandez-Rivera *et al*, 2003). However, changes in size can cause body composition artefacts in DXA data and therefore also need to be explored.

Carvalho *et al*'s systemic review of aBMD in HIV-positive women taking ART focused on 5 studies published between 2001 – 2009, using DXA to evaluate aBMD, concluded that femoral neck aBMD was more than 3% lower in women on PI-based-ART (PI-ART). When comparing women using PI-ART and not using PI-ART (i.e. non-PI-based-ART) there was no difference noted at the lumbar spine (Carvalho *et al*, 2010). Four out of five of these studies were cross sectional and conducted in the USA and one examined data from North America, UK, France, and Spain. The one randomised study of different ART regimes (Brown *et al*, 2009) did not have a HIV-negative control group (see Figure



2-21). None examined bone loss in women in the developing world, although the majority of women were classified as non-white, i.e. of "African American or Hispanic origins" (Carvalho *et al*, 2010).

**Figure 2-21 Systematic review of HIV and bone health studies**

**Table 1.** Selected studies: location, language, study design and objectives

| Author/year | Location | Language | Study design/follow up | Objectives |
|---|---|---|---|---|
| Brown and cols. (38), 2009 | United States, Canada, France, Spain and United Kingdom | English | Randomized Clinical trial open label/96 weeks | Evaluate changes in BMD prospectively in antiretroviral-naive persons initiating a PI *versus* a NNRTI-based regimen; determine whether removal of ZDV/3TC backbone impacts BMD; identify factors associated with BMD loss over 96 weeks, including systemic inflammation |
| Anastos and cols. (28), 2007 | United States | English | Cross-sectional | Verify association of BMD with HIV infection and HIV therapy in women enrolled in the Women's Interagency HIV Study (WIHS) |
| Yin and cols. (33), 2005 | United States | English | Cross-sectional | Assess risk factors for osteoporosis and measured BMD and biochemical indices of mineral metabolism in a group of HIV-infected post-menopausal women |
| Dolan and cols. (34), 2004 | United States | English | Cross-sectional | Investigate bone density and associated factors in normal weight, ambulatory HIV-infected women |
| Huang and cols. (35), 2001 | United States | English | Cross-sectional | Determine the risk factors for osteopenia in androgen-deficient women with Aids syndrome wasting |

BMD: bone mineral density; NNRTI: non-nucleoside reversal transcriptase inhibitor; ZDV: zidovudine; 3TC: lamivudine; HIV: human immunodeficiency virus; Aids: acquired immunodeficiency syndrome.

Summary table of systematic review of published studies HIV and aBMD (up to 2010) (Carvalho *et al*, 2010) [Reproduced with permission]

### 2.4.3 Effects of different classes of ART on bone health

Various different classes of ART have also been correlated with increased rates of osteoporosis and osteopaenia wherein ART-exposed HIV-positive patients had a 2.5-fold increased odds of low aBMD compared to those not exposed to ART (Tebas *et al*, 2000; Brown *et al*, 2009).

In an important study by Brown *et al* (2009), in a majority male population, n=106 aged 40 years or less, there were overall rates of whole body (WB) aBMD decline of -2.3% (NNRTI) and -2.5% (PI) over 96 weeks on ART; 15% of patients in both treatment arms lost >5% aBMD. This higher rate of loss was positively associated with lower $CD_4$ count, higher fasting glucose, and non-black race (Brown *et al*, 2009). These figures compare with estimated annual aBMD losses of 0.5-1% in adults aged over 35 years (Powderly *et al*, 2005) and 1-2% loss in the early postmenopausal years (Hodgson *et al*, 2003).

Mallon *et al* studied 40 HIV-positive, treatment-naive men, just beginning ART. There was an initial rise in WB aBMD after starting ART up to 24 weeks afterwhich the T-score

fell below that at baseline by week 144. Whole body DXA T-scores <-1.0 in these ART initiated men increased from 13% at baseline to 22% at week 144 (Mallon *et al*, 2003). The utility and clinical relevance of using WB aBMD T-score as a measure of bone loss is questionable because WB DXA is not used to diagnosis osteoporosis or response to anti-resorptive treatment.

Dolan *et al* followed up 100 female patients in Boston, USA along with age-, BMI- and race-matched controls (mean age 41 years) for two years. Historical low weight, duration of NRTI use and low follicle stimulating hormone (FSH) concentrations were positively associated with low LS aBMD. Duration of HIV infection, BMI, historical low weight, smoking, N-terminal telopeptides of type-I collagen (NTx), HIV viral load, osteocalcin and low plasma 25(OH)D were positively associated with low total hip (TH) aBMD at baseline. Whilst aBMD remained lower in HIV-positive women than controls, despite controlling for BMI, race, and menstrual function, the rate of aBMD loss was not different in cases and controls. In the HIV-infected group longitudinal aBMD loss was not associated with ART use but baseline aBMD, $CD_4$ count, and NTx (Dolan *et al*, 2006).

#### 2.4.3.1 Nucleoside reverse transcriptase inhibitors

Nucleoside reverse transcriptase inhibitors (NRTI) are the most common class of ART used worldwide. Older studies have found cumulative NRTI use to predict decreases in aBMD. However, cumulative NRTI use may be a surrogate for duration of HIV infection rather than represent an independent risk factor (Gold *et al*, 2002). Other studies have examined the role of thymidine analogue NRTIs in the aetiology of reduced aBMD. The thymidine analogues stavudine (d4T) and zidovudine (AZT) are associated with metabolic complications, particularly the lipodystrophy syndrome (Mallal *et al*, 2000), as well as the development of decreased aBMD. In vitro and an in-vivo mouse model data have demonstrated that AZT stimulates osteoclasts (Pan *et al*, 2006). A further indirect effect of thymidine analogues may be mitochondrial toxicity that results in lactic acidaemia (Shahmanesh *et al*, 2002) and, consequently, reduced aBMD via disruptions in acid-base homeostasis (Carr *et al*, 2001; New, 2002).



#### 2.4.3.2 Tenofovir

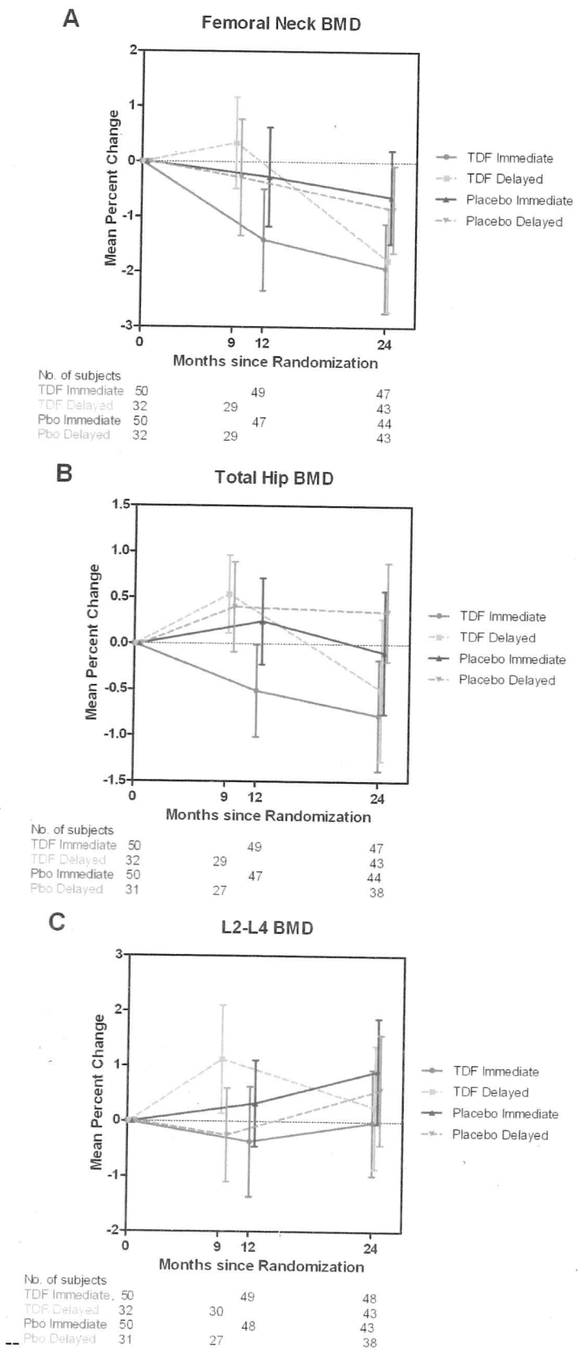
The NRTI tenfovir (TDF) is the ARV most associated with bone loss and has also been shown to reduce aBMD. The large "Gilead 903 study" showed that patients receiving tenofovir had significant decreases in aBMD at 144 weeks. Compared with thymidine analogues those receiving tenofovir had a -2.2% loss of LS aBMD vs. -1.0% ($p$=0.001, between groups) with non-significant differences at the hip (-2.8% tenofovir, -2.4% stavudine, $p$=0.06, between groups). These changes occurred during weeks 24 – 28 and then plateaued to week 144 (Gallant *et al*, 2004). The mechanism by which tenofovir induces bone loss is not fully understood but seems to be a consequence of its action on the proximal renal tubule, increasing phosphate wasting (Woodward *et al*, 2009). Macaques treated with high-dose tenofovir develop an osteomalacia-like appearance on bone histological examination (Castillo *et al*, 2002). It is also thought that tenofovir may have a direct inhibitory effect on renal 1-alpha hydroxylase thereby decreasing $1,25(OH)_2D$ and, hence, disrupting calcium/phosphate homeostasis (Brown *et al*, 2006a).

A fascinating insight into the effects of tenofovir is provided by the 2011 trial by Liu *et al* which measured longitudinal aBMD change in HIV-negative men using daily tenofovir (vs. placebo) as part of a HIV-prevention strategy, the so-called Pre-Exposure Prophylaxis (or PrEP). In the intention to treat (ITT) longitudinal analysis, there was a 1.1% net decrease in mean aBMD in the tenofovir vs. the placebo group at the femoral neck (95% CI 0.4-1.9% $p$ = 0.004), 0.8% net decrease at the total hip (95% CI 0.3% $p$ = 0.003), and 0.7% decrease at the LS (L2-L4) (95% CI -0.1-1.5% $p$ = 0.11). At baseline, there were higher than expected rates of low aBMD in these men and by 24 months, 13% vs. 6% of participants had experienced >5% aBMD loss at the femoral neck in the tenofovir vs. placebo groups (p = 0.13) (see Figure 2-22). These changes were most marked in the first 12 months of tenofovir exposure (Liu *et al*, 2011) and are in keeping with data from Hansen *et al* and McComsey *et al*, which demonstrated that most ART-related bone loss occurs in the first 12 months of exposure (Hansen *et al*,

2011; McComsey *et al*, 2011), although other authors have not reported bone loss during the same period (Bolland *et al*, 2012b).



**Figure 2-22 aBMD loss after exposure to tenofovir**




(Liu *et al*, 2011) [Content under open content licenses may be reused without any need to contact the licensor. See: http://creativecommons.org/licenses/by/2.5/]

A bone sub-study of the 'ACTG A5202' trial showed that tenofovir is associated with greater bone loss than other NRTI treatment as demonstrated in the 'A5224s' trial (see Section 2.4.2). In this trial of ART-naïve patients, at week 96, the mean percentage changes from baseline in spine and hip aBMD for Abacavir-lamivudine (ABC-3TC) versus tenofovir-emtricitabine (tenofovir-FTC) were -1.3% and -3.3% ($p$= 0.004) and -2.6% and -4.0% ( $p$= 0.024), respectively (McComsey *et al*, 2011).

#### 2.4.3.3 Protease inhibitors

An important early cross sectional study by Tebas *et al* (Tebas *et al*, 2000) demonstrated that men receiving PI-based ART had a relative risk of osteoporosis and osteopaenia of 2.19. Half of men on PI/ART were classed as osteoporotic or osteopaenic compared with non-PI/ART and controls. However, the men on PI were significantly older and other important factors such as vitamin D status, and alcohol or steroid use were not reported (Tebas *et al*, 2000). A cross-sectional study by Bruera *et al* (Bruera *et al*, 2003) comparing HIV-infected patients on different ART regimes and HIV-negative controls found no difference in rates of low aBMD by ART class (PI vs. non-PI) but noted a significantly lower proportion with a T-score of greater than -1.0 in HIV-infected patients compared with controls at femoral neck (35 – 54% and 58% respectively) and lumbar spine (19 – 31% and 85% respectively) (Bruera *et al*, 2003). More PI studies have confirmed Tebas' early finding (Amiel *et al*, 2004; Brown *et al*, 2004). Amiel *et al* examined 148 HIV-positive men, mean age 40 years and 81 HIV-negative controls; 100/148 of the HIV-positive men were receiving ART. Of HIV-positive men, 16% had any site Z-score less than -2.5 compared with 4% of controls. Low BMI and raised bone resorption markers were positively associated with low aBMD irrespective of ART use (Amiel *et al*, 2004).

In a review article by Pollock *et al*, those treated with a PI compared to those treated with a non-PI-based ART regime had increased odds of reduced aBMD and osteoporosis (1.5 and 1.6 respectively). However, since aBMD assessment did not generally predate the use of ART, the low aBMD may reflect the effects of HIV infection rather than choice



of ART (Pollock *et al*, 2009). In a French trial by Duvivier *et al* (Duvivier *et al*, 2009) patients were randomised to receive different ART regimes, and those assigned to either of the PI-based arms (PI/NNRTI or PI/NRTI) had the greatest percentage decrease in LS aBMD at 48 weeks compared to baseline when compared to those in the NNRTI/NRTI arm (-4.4%, -5.8%, and -1.5% respectively). This contrasts with a study by Brown *et al* that did not show a difference in loss of Whole Body aBMD in PI vs. non-PI treated patients (Brown *et al*, 2009), though these data are not directly comparable with LS aBMD. A potential explanation for the close correlation between PI use and lower aBMD is suggested by the in vitro data linking PIs and increased osteoclastogenesis and decreased osteogenesis via direct inhibition of osteoprotegerin (OPG) (Pan *et al*, 2006). What is more, in ex-vivo experiments, different PIs have opposing effects: indinavir increases bone loss by effects on osteoblast recruitment, and ritonavir blocks osteoclastogenesis, reducing bone loss. The authors concluded that their results "suggest that bone and fat formation … may be coordinately down-regulated by some but not all PIs" (Jain *et al*, 2002).

PI-based ART study results may also contradict one another because of different study designs, and because of a difference in the PIs employed. Also, since PIs are often used together and in combination with NRTI, it is difficult to separate the individual drug effects (Pan *et al*, 2006; Avenell *et al*, 2012). Because data on the effects of particular ART on aBMD are so conflicting there is a need for further investigation. The lack of consistency across trials and studies suggests that there is a complex, multifactorial association between specific PI treatment and bone disease, and for which studies have not yet been well-enough designed to elucidate.

#### 2.4.3.4 Non-Nucleoside Reverse transcriptase inhibitors

The balance of evidence suggests that NNRTI use has less detrimental skeletal effects than PIs. PI-based regimes tend to be reserved for those with previous virological failure, and therefore are used in patients with longer duration of infection; also it is impossible to factor out the effects of HIV infection in these studies. A study of PI vs.

NNRTI based regimes in HIV-positive young adults/adolescents and adults (Mulligan *et al*, 2012) recruited 199 HIV-positive men, of these 52 were taking NNRTI-based and 42 PI-based ART. These were compared with 53 HIV-negative controls; the subjects were aged between 14 – 25 years. The study demonstrated that those exposed to ART had lower mean aBMD and Z-scores than controls with the lowest aBMD in the PI-exposed group. The bone sub-study of 'ACTG A5202' also demonstrated a less marked loss of bone with efavirenz (NNRTI) than Atazanavir/ritonavir (PI). Because this was a RCT, there was less inherent bias potentially seen in observational studies. At week 96, the mean percentage changes from baseline in spine and hip aBMD for efavirenz vs. atazanavir/ritonavir were -1.7% and -3.1% ($p$= 0.035) and -3.1% and -3.4% ($p$= 0.61), respectively. These results could also be interpreted as not so much a negative effect of these two regimes, but rather as NNRTI 'protecting' against spine but not hip aBMD loss (McComsey *et al*, 2011).

## 2.5 HIV/ART and vitamin D

There are complex interactions between HIV infection, risk of osteoporosis and fracture, and poor vitamin D status. In a review, Brown and McComsey (Table 2-4) listed potential factors associated with low aBMD in patients with HIV infection and describe HIV-associated bone loss in terms of ART medication and viral factors. These included ART, HIV, and patient factors. Patient factors are those that may, or may not, be related to HIV-status. Some, e.g. smoking, alcohol use and poor vitamin D status, which are present in the general population may be more prevalent in HIV-positive patients, at least within developed countries (Mondy *et al*, 2003; Dolan *et al*, 2006; Prior *et al*, 2007).



**Table 2-4 Risk factors for reduced aBMD in HIV infection**

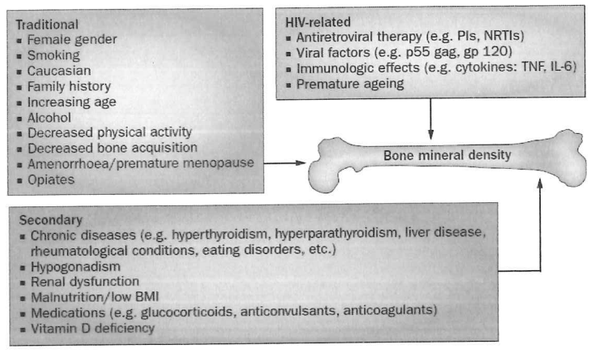
| |
|---|
| **Antiretroviral medications:** |
| Proteases inhibitors (PI) * |
| Reverse transcriptase inhibitors |
| Thymidine analogues* |
| Tenofovir |
| **HIV disease factors:** |
| Duration of infection |
| HIV disease severity |
| Viral load |
| **Patient factors:** |
| Low body weight/body mass index (BMI) |
| Smoking |
| Hypogonadism |
| Vitamin D deficiency |
| Glucocorticoid use |
| Body fat changes* |
| Immobility |
| Drug and alcohol misuse |
| Race |
| Sex |
| Age |
| * controversial |

Adapted from Brown and McComsey (Brown *et al*, 2006a)

In a 2012 review, Walker-Bone highlights the HIV-specific, secondary and traditional risk factors for HIV-associated low bone mass, which illustrates the different potential negative effects of these factors on aBMD (Figure 2-23). What remains unknown is if HIV infection may make individuals more susceptible to certain 'patient' or 'secondary' factors e.g. poor vitamin D status.

HIV and ART, separately, and in combination have been associated with poor vitamin D status. In addition, other drugs commonly used in HIV-positive patients may have deleterious effects on vitamin D status (see Section 2.3). For example, potent CYP450-inducing drugs, such as rifabutin (used to treat TB), can induce osteomalacia via its catabolism of 25(OH)D in the liver (Bolland *et al*, 2008). There may also be a cumulative, negative effect on vitamin D status of using drugs such as rifabutin in combination with ART.

**Figure 2-23 Traditional, secondary, and HIV-related effects on aBMD**




(Walker-Bone, 2012) [Reproduced with permission]

Seminari *et al* described an observational study of 68 mainly male Italian patients with a median age of 41 years. Of these patients, 81% had 25(OH)D <45nmol/l (18ng/ml), with secondary hyperparathyroidism in 26%, which negatively correlated with 25(OH)D as expected. However there were no healthy controls in the study (Seminari *et al*, 2005). In a 2010 paper, Mueller *et al* describe a prevalence of 25(OH)D of <30nmol/l in 42% of their Swiss HIV-infected patients in spring months (Mueller *et al*, 2010).

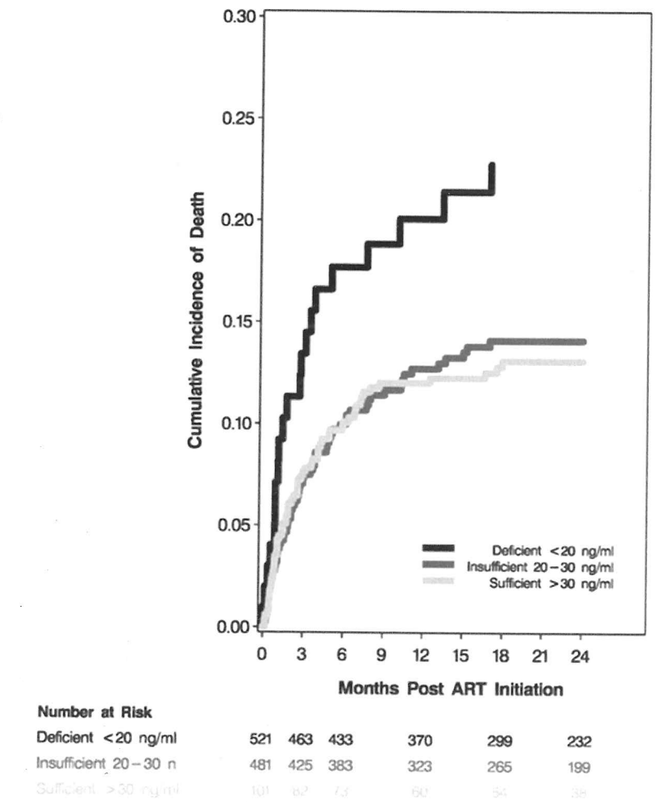
In their 2012 paper, Sudfeld *et al* observed that

> "To date no longitudinal studies of vitamin D and HIV disease progression have been conducted among adults receiving ART in sub-Saharan Africa or for men and non-pregnant women in a resource-limited setting. Examining the relationship between vitamin D and HIV disease progression in this population is essential given over one million adults initiated ART in sub-Saharan Africa in 2010 and coverage is rapidly expanding. Furthermore, no studies have investigated the impact of vitamin D on change in $CD_4$ T-cell count in HIV-infected individuals receiving ART in a resource-limited setting where individuals start ART at low $CD_4$ T-cell counts, which will give valuable insight to the mechanism of vitamin D on mortality."



The authors conducted a prospective cohort study of Tanzanian adults initiating ART to address these specific issues and concluded that "deficient (defined as <50 nmol/l) vitamin D levels may lead to increased mortality in individuals receiving ART and this relationship does not appear to be due to impaired $CD_4$ T-cell reconstitution" (Sudfeld *et al*, 2012) (Figure 2-24).

**Figure 2-24 Vitamin D status and mortality**




Relationship between incidence of death and vitamin D status, post ART-initiation (Sudfeld *et al*, 2012) [Content under open content licenses may be reused without any need to contact the licensor. See: http://creativecommons.org/licenses/by/2.5/]

Mehta *et al*'s 2010 paper from Tanzania found that in HIV-positive pregnant women, not in receipt of ART, those with the highest concentrations of serum 25(OH)D, mean value was 107 ±23 nmol/l, had a 42% less all-cause mortality when compared with those in the lowest quarter of vitamin D status (Mehta *et al*, 2010). A large international study,

with data from 1985 participants, from Europe, Israel, and Argentina, described HIV-positive patients >73% male, and >80% white (most of whom were receiving ART) with the highest vitamin D status (25(OH)D >50nmol/l) having 0.56 times rates of mortality compared with those in the lowest third (<30nmol/l) (Viard *et al*, 2011).

A 2008 Dutch study demonstrated low 25(OH)D concentrations (<25nmol/l) in 29% of 252 patients with HIV infection, during the winter months (Van Den Bout-Van Den Beukel *et al*, 2008a) but there was not a HIV-negative control group to compare these to. Certain classes of ART were more associated with low 25(OH)D concentrations than others; specifically NNRTI compared to PI-containing ART. In this study, patients dark skin colour was the only other independent risk factor for low 25(OH)D.

Other markers of vitamin D metabolism, such as $1,25(OH)_2D$, have been studied in an attempt to assess the correlation between vitamin D metabolites and HIV-associated prognosis. A study carried out before the advent of modern combination ART demonstrated the positive correlation between symptomatic HIV disease, reduced survival, reduced $CD_4$ count, and low plasma $1,25(OH)_2D$ concentrations (defined as <25pg/mL) compared with HIV-negative controls and those without symptoms. Interestingly low $1,25(OH)_2D$ concentrations were more closely correlated with reduced survival than even $CD_4$ count, which is the most commonly used proxy measure of HIV disease progression. The mechanisms for this effect are not known but it is speculated that it may be the result of altered antimicrobial activity by the cellular immune system, which utilises $1,25(OH)_2D$ to effect some of its antimicrobial effects (Haug *et al*, 1994). This, with other studies (Aukrust *et al*, 1999), provides an intriguing insight into the interaction between HIV infection and vitamin D metabolism and HIV disease progression. It suggests that HIV, possibly along with AIDS-related conditions, interferes with renal vitamin D hydroxylation or $1,25(OH)_2D$ consumption or catabolism (e.g. metabolism to $1,24,25(OH)_2D$), and a potential interplay between raised TNFa and depressed $1,25(OH)_2D$ concentrations. As intracellular $1,25(OH)_2D$ has been shown to enhance the effect of killing of mycobacteria in human macrophages, Haug *et al*



speculate that low plasma $1,25(OH)_2D$ may contribute to impaired anti-mycobacterial defence thereby predisposing HIV-positive patients to mycobacterial infections (Haug *et al*, 1994).

The mechanism for this observed low plasma $1,25(OH)_2D$ is elusive as 25(OH)D is taken up by immune cells and converted into $1,25(OH)_2D$. Therefore the process resulting in depleted plasma $1,25(OH)_2D$ may be distinct from its intracellular action. Low plasma $1,25(OH)_2D$ may not reflect the intracellular environment, so it cannot be assumed that low plasma concentrations equate to low intracellular concentrations, with attendant suppressed antimicrobial effects. A study in the post-ART era examined $1,25(OH)_2D$ concentrations and it was also noted that they are low; and seen in conjunction with low PTH, and low or normal calcium, however, vitamin D status was not described. It was speculated that the low observed $1,25(OH)_2D$ was due to the inhibitory effects of TNFa on PTH secretion and renal hydroxylation of 25(OH)D to $1,25(OH)_2D$ (Aukrust *et al*, 1999).

#### 2.5.1.1 Vitamin D supplementation in HIV infection

In small trials, vitamin D supplementation in HIV-positive adults (n=20 – 74) (Van den Bout-van den Beukel *et al*, 2008b; Childs *et al*, 2009) and children (n=29) (Arpadi *et al*, 2009) has been evaluated. These have demonstrated short-term safety, by not inducing hypercalcaemia, and efficacy by increasing 25(OH)D concentrations from baseline. One study included daily doses of vitamin D3 of up to 2800 IU/d (70 mcg/d) for HIV-positive men with initial concentrations of 25(OH)D of <25 nmol/l (Childs *et al*, 2009) without toxicity.

Small-scale studies in HIV-positive patients to assess the effect of supplemental vitamin D on bone health have been carried out. In an open label, non-randomised Dutch study, after 48 weeks of vitamin D supplementation (1000 - 2000 IU/d (25 – 50 mcg/d)) there was no significant difference in whole body aBMD. However the mean femoral neck T-score was significantly lower at 48 weeks suggesting on-going loss at this site despite supplementation with vitamin D (Van den Bout-van den Beukel *et al*, 2008b). This may

suggest that loss of bone mineral was independent of vitamin D status or that a suboptimal dosing schedule was used. In this study, vitamin D supplementation resulted in decreases in PTH and increases in 25(OH)D and $1,25(OH)_2D$, but when the dose was reduced from 2000 to 1000 IU/d after 14 weeks, as per study design, only 25(OH)D was significantly raised from baseline. Supplementation had no effect on $CD_4$ count or HIV viral load at either dose (Van den Bout-van den Beukel *et al*, 2008b).

#### 2.5.1.2 ART and vitamin D metabolism interactions

Many enzymes of the cytochrome P (CYP) 450 family involved in vitamin D metabolism are thought to interact with both NNRTI and PI drugs. Examples are hepatic CYP27A, CYP2R1, and CYP3A4 enzymes involved in 25-hydroxylation and renal CYP27B1 in 1,25-hydroxylation (see Sections 2.3.8 and 2.3.3). There are also in vivo demonstrations of the inhibitory effects of the PIs ritonavir, indinavir, and nelfinavir on CYP450 enzymes with net decreases in 25(OH)D in studies that have associated ART exposure with poor vitamin D status (Figure 2-25). In 211 HIV-positive patients in Switzerland (Mueller *et al*, 2010), NNRTI use was associated with lower 25(OH)D concentrations and tenofovir use associated with higher $1,25(OH)_2D$ concentrations. This study retrospectively assessed 25(OH)D status of 211 HIV-infected patients at baseline, pre-ART and 12 and 18 months post-ART initiation. Only data from patients who were strictly virologically suppressed were analysed; the study was limited by lack of a control group, and dietary or supplementary vitamin D intake data. The level of 'sufficiency' was set at >75nmol/l. The patients were predominantly white (88%) and male (75%). 43% were treated with NNRTI and 53% a PI-based ART regime. As anticipated 25(OH)D concentration was higher in autumn after peak sun exposure than in spring (post winter), but not significantly affected by ART. 'Sufficient' 25(OH)D concentrations (>75nmol/l) were reached in 4% in the autumn compared with 5-10% measured in the spring. Black patients did not demonstrate increased 25(OH)D concentrations in the autumn compared with 'non-black'. In multivariate analysis NNRTI use was associated with a significant decrease in 25(OH)D concentrations from 46 (IQR 30-67) to 39 (IQR 21-60) nmol/l; PI use was associated with a non-significant rise from 42 to 43 nmol/l. It was suggested



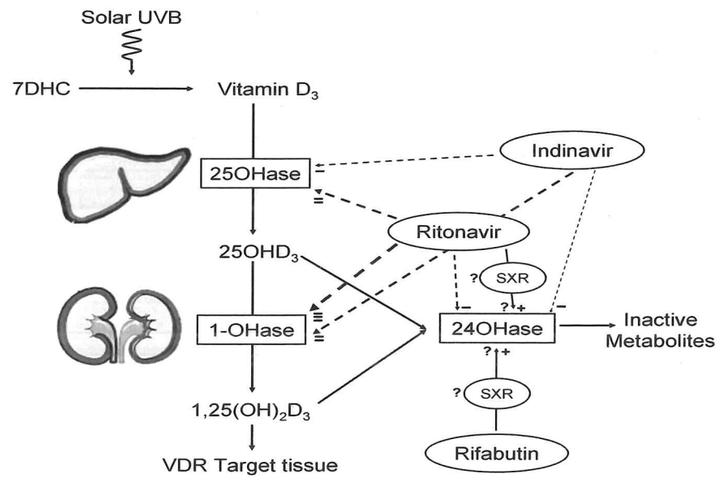
that 6-15% of the general Swiss population have 25(OH)D concentrations <75nmol/l during winter (Mueller *et al*, 2010).

Several enzymes have been associated with the hydroxylation of 25(OH)D, including mitochondrial CYP27A and microsomal CYP2R1 (Wikvall, 2001), the 1-alpha-hydroxylase CYP27B and the 24-hydroxylase CYP24 (Pascussi *et al*, 2005). CYP3A4 has been demonstrated to be a human microsomal vitamin D 25-hydroxylase (Gupta *et al*, 2004), efavirenz is a potent inducer of this enzyme and reduces the plasma area under the curve (AUC) of other CYP3A4 metabolised drugs, such as methadone, by >50%. CYP3A4 is important clinically because it is the most abundant CYP enzyme and is involved in the metabolism of more than half of the drugs currently on the pharmaceutical market as well as potential effects on vitamin D metabolism. Therefore, efavirenz, one of the most commonly used ARV, may act in two distinct ways: limiting the 'supply' of 25(OH)D (via CYP3A4 in the liver) and increasing the 'breakdown' of $1,25(OH)_2D$ (via CYP24) (Gyllensten *et al*, 2006).

Tenofovir may induce hyperparathyroidism through effects on renal phosphate handling (Childs *et al*, 2008; Rosenvigne *et al*, 2010). As well as inducing renal hydroxylation pathways it is also possible that some ARV induce the hepatic and gastrointestinal cytochrome enzyme CYP450 3A4. Both commonly used NNRTI ART (efavirenz and nevirapine) induce CYP450 3A4, mediating degradation of $1,25(OH)_2D$ (Xu *et al*, 2006). In a case report, Gyllensten had described an African male patient in Sweden (59ºN) developing undetectable (i.e. <18nmol/l) 25(OH)D 18 months after commencing efavirenz-containing ART. This was associated with raised PTH and alkaline phosphatase (ALP) and low LS and FN aBMD by DXA (T-score -3.13. and -3.84 respectively). All parameters improved after treatment with calcium and vitamin D supplementation; after nine months of which aBMD had improved by 4% at the LS and 11% at the femoral neck (Gyllensten *et al*, 2006).

Interest in the relative roles of different classes of ART on vitamin D metabolism continues. As described above some studies suggest an association between PI and others to NNRTI use, and low plasma 25(OH)D concentrations (Cozzolino *et al*, 2003; Gyllensten *et al*, 2006; Brown, 2009). Both PI and NNRTI have potent enzymatic effects that result in complex and often idiosyncratic effects on drug concentrations in particular. It has been suggested that PIs not only reduce $1,25(OH)_2D$ production by suppressing 25-and 1- alpha hydroxylases but also mildly suppressing 24-hydroxylase and therefore have comparatively less influence on the catabolism of $1,25(OH)_2D$ (Cozzolino *et al*, 2003; Holick, 2007; Bolland *et al*, 2008). A similar effect on cytochrome P450 has been mooted as responsible for inhibition of oestrogen-synthesizing aromatase thereby contributing further to ART-related bone loss through oestrogen depletion (Cozzolino *et al*, 2003) (see Section 2.4.1).

**Figure 2-25 Interaction of ART with vitamin D.**




Ritonavir and Indinavir (PIs) have increasing degrees of inhibition as indicated by –, = etc. Stimulatory affect indicated by + sign.
Rifabutin is an antimicrobial agent with enzyme induction affects. SXR = steroid and xenobiotic receptor

(Bolland *et al*, 2008) [Content under open content licenses may be reused without any need to contact the licensor. See: http://creativecommons.org/licenses/by/2.5/]



## 2.6 Micronutrient intakes related to HIV, vitamin D, and bone health

In South Africa there are high rates of under- and over- nutrition, so macronutrient intake is an important area of research for general health. However since the focus of this thesis is bone health and HIV this section will focus on micronutrient intake, particularly those relating to bone health and HIV infection. Some micronutrients such as calcium, phosphorus, boron, and magnesium are important for bone health and others, such as zinc, are important for immune function and bone formation. The sources of calcium and phosphorus in the diet have been described in Section 2.3.8.

Villamor suggests that 90% of HIV-infected people live in areas of the world where nutritional deficiencies are prevalent (Villamor, 2006). Data on the role of micronutrient deficiencies and the use of supplementation are well described in the context of HIV infection, largely in Western populations (Coutsoudis *et al*, 1995; Tang *et al*, 1996; Baum *et al*, 1997). In the pre-ART era, studies examined the role of different micronutrients on HIV-associated mortality. In a US study by Tang *et al* (Tang *et al*, 1996) data were collected at baseline in 1984 via a semi-quantitative food frequency questionnaire, and micronutrient intakes were correlated with subsequent mortality in an eight-year follow-up. The highest quarter of B group vitamins intake was positively associated with improved survival, whereas the highest zinc intake was associated with poorer survival; a finding that appears counter intuitive given zinc's role in immune function. This suggests that there is not always a predictable correlation between micronutrient intakes and health outcomes. High intakes of some nutrients, such as iron, may decrease host survival by providing disease-causing microorganisms with essential nutrients for growth (Smith *et al*, 1989). On the other hand, Allard has described the favourable effects of supplements containing vitamins C and E in HIV-infected patients on plasma viral load (Allard *et al*, 1998). This was a Canadian double blind RCT in which patients (n=49) were randomised to receive 1000 mg of vitamin C and 800 IU of alpha-Tocopherol per day versus placebo. However, more recently well-conducted supplementation studies have associated high dose multivitamin supplements with

increased liver function abnormalities in Tanzanian HIV-positive patients (Isanaka *et al*, 2012), as well as clinical mastitis and increased HIV-transmission via breast milk (Arsenault *et al*, 2010; Villamor *et al*, 2010). Such data challenge the validity of the notion that high-dose supplements improve outcomes even in populations likely to be habituated to chronic nutrient insufficiency. Because ART remains limited in many parts of the world, low-cost, health-promoting interventions, including nutritional supplementation, to decrease communicable and non-communicable diseases may have an important role for those who are unable to access ART or who do not yet require it (Fawzi *et al*, 2004). Clearly, studies in different population groups need to be carried out before general or specific recommendations about nutrient supplementation can be made.

## 2.7 Concluding remarks

A consensus is evolving linking HIV infection and ART exposure with premature osteoporosis (Grund *et al*, 2009; Nachega *et al*, 2009), "sounding the alarm that we may expect an epidemic of fragility fractures in the future" (Amorosa *et al*, 2006).

When considering the interplay between vitamin D status, bone health, and communicable disease, such as HIV infection, it is important that populations are studied when undertaking their habitual activities and habits (which might include supplement use). This is particularly pertinent in tropical countries where it is easy to assume that vitamin D status is not affected by season and vitamin D deficiency will be absent. In subtropical African countries, such as South Africa, there are distinct seasons with a cold winter during which there is decreased or absent UBV radiation and people tend to cover their skin to protect against the cold, thereby reducing their opportunity for vitamin D synthesis (Pettifor *et al*, 1978; Pettifor *et al*, 1996).

In parts of the world where the HIV epidemic is evolving along with a shift from under- to over-nutrition such as in South Africa, and ongoing obesity epidemics such as in the USA, the possibility of permanent sequestration of plasma 25(OH)D into adipose tissue, obesity per se (Wortsman *et al*, 2000; Lenders *et al*, 2009) and HIV-associated



lipodystrophy may be important factors in determining vitamin D status in HIV-positive patients. Whilst these findings might not be applicable to all patients, they do suggest that there is a need to conduct studies that will answer the questions: is poor vitamin D status a problem in particular populations? If so, would vitamin D supplementation in HIV-positive individuals, both on and off ART, improve measures of morbidity and mortality as well as bone health? At present there are a lack of RCT data supporting the routine supplementation of HIV-positive individuals, particularly those commencing ART.

As discussed (see Section 2.2.7.5), historically, some black African populations have lower rates of fracture, however the protective effect African ethnicity seems to afford against fracture may be reduced in populations with high rates of HIV infection. If HIV and/or ART results in negative effects on muscle function, peripheral nerves, the autonomic nervous system as well as bone, all of which are important contributors to fracture risk, the fracture rates may increase in these populations. So it is important to describe aBMD in populations who may be at increased fracture risk (HIV-positive women), particularly in those with less well defined skeletal phenotypes (black Africans). Once described, preventative measures can be investigated if appropriate.

# 3 Study design

The aims of the study were detailed in Section 1.5:

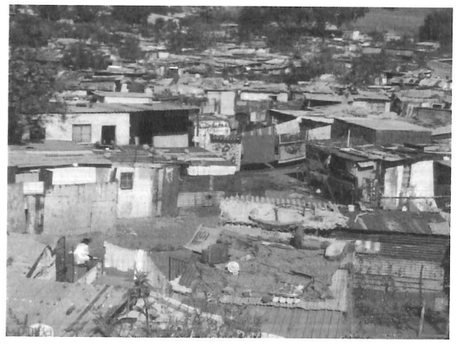
1. To compare baseline bone mineral status in ART-naïve HIV-positive South African women and compare with HIV-negative controls. To compare baseline anthropometry, body composition and serum concentrations of 25(OH)D in HIV-positive women and HIV-negative women;

2. To evaluate the changes in bone mineral status, anthropometry, body composition and vitamin D status in HIV-positive South African women with preserved and low $CD_4$ counts with controls, at 12 months after baseline; and to compare those HIV-positive women who progressed to ART during the year with those who did not.

## 3.1 Study location

The study took place at the Developmental Pathways for Health Research Unit (DPHRU), which is a joint MRC (South Africa) and the University of the Witwatersrand unit. DPHRU has two main sites, one at the University of the Witwatersrand's Medical School in central Johannesburg and the other at the Chris Hani Baragwanath Hospital in Soweto, approximately 13 kilometres south of central Johannesburg (see Figure 3-1). All reference to DPHRU pertains to the Soweto site.

**Figure 3-1 Soweto**



[Source: https://www.google.co.uk/search?hl=en&q=soweto+south+africa&tbm]



DPHRU was formally established in 2011 after reconfiguration of the previous Mineral Metabolism Unit and is host to the longitudinal birth cohort, the Birth to Twenty Study (Richter *et al*, 2007) (see Figure 3-2).

**Figure 3-2 The DPHRU building at the Chris Hani Baragwanath Hospital**

The Chris Hani Baragwanath Hospital is the only government hospital providing care to the population of Soweto (see Figure 3-3). This comprises approximately 1.3 million individuals, depending on time of year and seasonal changes in internal migration. The hospital is mostly used by the most disadvantaged patients for their health needs. Poverty is a major problem, with high rates of HIV infection, unemployment, and low educational attainment (Willey *et al*, 2009; Guateng: City of Johannesburg, 2013).

Figure 3-3 The Chris Hani Baragwanath Hospital




## 3.2 Setting up the study in South Africa

After planning discussions with potential collaborators in South Africa I travelled to Johannesburg in March/April 2010 during which time I met several key collaborators; these included Professors John Pettifor and Shane Norris at the University of the Witwatersrand. I also had meetings with Dr Neil Martinson at the Perinatal HIV Research Unit (PRHU) at the University of the Witwatersrand and Dr Alan Karstaedt, Head of Infectious Diseases at the Chris Hani Baragwanath Hospital. These four individuals were very supportive of the project and expressed a wish to be involved in the collaboration. Initial ideas about study design were revised and improved after this trip. Development of the study benefitted from close collaboration with laboratory and technical staff at the DPHRU facilities in Johannesburg; and this was agreed as the site for the study. Logistical considerations were discussed and incorporated in the study design (e.g. provision of transport costs). It was suggested that in addition to the PhD project that a follow-up of two and three years be incorporated in the study protocol to ultimately allow for the description of longer-term changes in bone and vitamin D status.

## 3.3 Study protocol: submission to Ethics and Scientific Committees

After approvals within MRC Human Nutrition Research, the protocol was submitted to MRC HNR Scientific Coordinating Committee (SCC) and to the University of the Witwatersrand Ethics Committee for scientific and ethical approval (Ethics Committee reference: HREC Number, M101525). The study protocol and supporting documentation were also submitted to, and approved by, the Gauteng Department of Health (see Appendix 8); this is required for all studies involving patients in this province. Approvals included scrutiny of patient-held records for clinical information if these were available. During the study, 4 minor amendments were made to the study design and successfully submitted to the University of the Witwatersrand Human Research Ethics Committee.

## 3.4 Study design

The study enrolled urban South African adult, premenopausal women who were over 18-years old.

The women were assigned to one of 3 groups:

- HIV-negative controls (Nref);
- HIV-positive with preserved $CD_4$ counts (≥350 $x10^6$/l) (Ppres);
- HIV-positive with low $CD_4$ counts (≤200 $x10^6$/l), due to start ART in the near future (Plow).

Nref had had recent (within 12 weeks) HIV tests, showing them to be negative. The women entered Ppres or Plow depending on the $CD_4$ count criteria set using South African eligibility thresholds for commencing ART. Local clinic staff sometimes commenced a woman on ART at a higher $CD_4$ count depending on clinical judgement.

The justification for the target numbers in each group is described in Section 3.5. Subjects were selected based on meeting the baseline inclusion and exclusion criteria (Table 3-1 and Table 3-2). An exclusion criterion was current or planned pregnancy. Women consented to undergo pregnancy testing at baseline and prior to each further study visit. The primary reason was to avoid recruiting women who would be ineligible to undergo DXA scanning. Lactating and postmenopausal women were not enrolled to avoid the confounding associated with rapid changes in skeletal mineralisation seen during lactation and the menopause. Women who became pregnant, who commenced lactating (following a pregnancy) or became climacteric after baseline remained in the study. Pregnant women did not undergo DXA scanning.

**Table 3-1 Study inclusion criteria**

| General baseline inclusion criteria: |
|---|
| No current AIDS defining illness (WHO or CDC definitions) |
| No known current chronic kidney, liver, thyroid, or bone disease |
| No current fracture (i.e. being actively treated) |
| Not currently pregnant, lactating or planning a pregnancy |
| No recent prolonged bed rest (i.e. >50% of day spent in bed for >2 weeks) |
| No use of the following medication:<br>- Steroids (i.e. >5 mg prednisolone/day (or equivalent) for >21 days in past 6 months. Inhaled and topical steroids were permitted)<br>- Bone specific medication (systemic chemotherapy in past 6 months, prior use of drugs to increase bone formation e.g. bisphosphonates)<br>- Drugs that may disrupt vitamin D metabolism, e.g. phenytoin |
| No complaints of persistent fever, cough, or severe pain in the past week. Other clinical or laboratory parameter deemed significant by study clinician |
| Not taking part in another research study |
| Expecting to be resident in Johannesburg area for 12 months post-enrolment |
| Black, premenopausal, and aged >18 years |
| Recent CD4 count if HIV-positive |



**Table 3-2 Study exclusion criteria**

| General baseline exclusion criteria: |
|---|
| Current AIDS defining illness (WHO or CDC definitions) |
| Known current chronic kidney, liver, thyroid, or bone disease |
| Current fracture (i.e. being actively treated) |
| Current pregnancy or lactation or planning pregnancy |
| Prolonged bed rest (i.e. >50% of day spent in bed for >2 weeks) |
| Use of the following medication:<br>- Steroids (i.e. >5 mg prednisolone/day (or equivalent) for >21 days in past 6 months. Inhaled and topical steroids were permitted)<br>- Bone specific medication (systemic chemotherapy in past 6 months, prior use of drugs to increase bone formation e.g. bisphosphonates)<br>- Drugs that may disrupt vitamin D metabolism, e.g. phenytoin |
| Complaints of persistent fever, cough, or severe pain in the past week. Other clinical or laboratory parameter deemed significant by study clinician |
| Taking part in another research study |
| Not expecting to be resident in Johannesburg area for 12 months post-enrolment |

The study was an observational longitudinal investigation. Details of the methods employed are provided in Chapter 4. At baseline, anthropometry, DXA scans, questionnaire data, and fasting blood and urine samples were collected. These were repeated at 6 and 12 months post baseline. Women in the Nref group were offered repeat HIV-testing at 6- and 12-month visits to ascertain current HIV status.

The study was designed so that the three study visits would be during summer (summer and autumn), winter (winter and spring) and summer (summer and autumn) in order to evaluate the effect of season, particularly on vitamin D status.

The study protocol was designed with a focus on subject safety and wellbeing. Strict inclusion and exclusion criteria (Table 3-1 and Table 3-2) were employed in order to enable timely access to ART and medical care for those HIV-infected women requiring ART-initiation. The exclusion criteria were intended to ensure that those with acute illness did not enrol in the study. Only those Plow women were recruited who did not exhibit advanced AIDS since this is associated with general frailty, profound weight loss, and associated effects on endocrine function and aBMD. In order to have immunologically similar groups, the initial upper $CD_4$ count limit for enrolment into Ppres was 500 $x10^6$/l, and the lower cutoff of 250 $x10^6$/l. A limitation of the study was that duration of HIV infection was generally not known as most HIV positive women had not

previously been tested. After the inception of the study and prior to completion of recruitment, the South African Department of Health amended the threshold for ART initiation from a $CD_4$ of ≤200 $x10^6$/l up to ≤350 $x10^6$/l (see Section 1.3.3). In order to be able to complete recruitment, an amendment was submitted to the Ethics Committee to allow greater flexibility in using $CD_4$ count cutoffs so that subjects could be recruited from a much larger pool of potential subjects. It was agreed that Ppres subjects would have a $CD_4$ count in the region of >350 $x10^6$/l and ideally ≤500 $x10^6$ /l wherever practical. Plow subjects would have $CD_4$ counts, wherever practical, in the region of ≤200 $x10^6$/l but ideally not <50 $x10^6$/l.

$CD_4$ and other clinical indicators for receiving ART followed prevailing South African guidelines and ART followed current South African standards of care. The study team did not initiate ART in the research clinic and encouraged all women to continue to attend their local clinical services for medical follow up.

To satisfy research and clinical governance, women gave written informed consent and were made aware that the study site did not provide clinical care; if clinical input was required during the course of the study, participants were referred to the appropriate facilities. Generic health advice was always offered to women if requested, and an experienced nursing sister (Thokozile Lephoto) was available to discuss social and general health issues. Those women who were initially in the Ppres group, who had an unanticipated fall in $CD_4$ count, or who developed symptoms of signs of progressing disease and so subsequently required ART initiation, were encouraged to seek medical care as soon as possible.

### 3.5 Sample size

Before this study the extent of low aBMD and rate of bone loss (as measured by DXA) and low serum 25(OH)D concentrations in this population were unknown. To detect a difference of 2% (0.4 SD) change in aBMD over 12 months, allowing for 5% SD, with 95% confidence and 80% power, 100 cases and 100 controls (n=200) were required. After discussion with a senior statistician (Dr Tony Fulford, MRC International Nutrition



Group), a more balanced study design across the three groups was adopted to better allow discrimination between the two HIV-positive groups. To this effect, and in order to account for losses to follow-up of 10%, the minimum number required in the HIV-positive group was increased to 146 whilst the number in the HIV-negative group was reduced to 95 (n=241).

These sample sizes would enable differences of 0.4SD to be detected in the primary outcomes as significant at 5% significance and 80% power. A difference of 2% change in lumbar spine aBMD over 12 months would provide a clinically pertinent measure of bone loss in this population of women. Such a rapid loss, associated with HIV infection and ART exposure would be analogous to aBMD loss in early menopause (1-2%) and would be measurable by DXA and clinically significant as a measure of increased future fracture risk.

In the SMART trial (see Section 2.4.2), Grund *et al* described an annual aBMD decline of 0.8% hip, 0.4% (spine DXA), and 2.4% (spine QCT) in the continuous ART group (Grund *et al*, 2009). Conversely other studies such as Dolan *et al*'s prospective cohort study, aBMD remained lower but stable in HIV-positive women compared with controls over two years (Dolan *et al*, 2006). Mondy *et al* described an increase in aBMD over the 72 week follow-up period in 90 patients. There was a small but statistically significant mean percentage increase in the subset of subjects who agreed to be followed up; at the LS this was 2.6 ±0.6%. This was independent of ART and HIV infection duration but was associated with an undetectable HIV viral load at baseline (Mondy *et al*, 2003). In this study>95% of subjects were already receiving ART at baseline so the relative effects of ART and other risk factors are difficult to disentangle.

In South Africa, HIV studies reported dropout rates between 2-10% (Maskew *et al*, 2007; MacPherson *et al*, 2009; Boulle *et al*, 2010). In this population of mobile, urban women we anticipated that loss to follow-up might be greater than the typical upper limit of 10% because of death, illness (particularly in HIV-positive subjects), and the potential

for relocation (away from Soweto). Since these women were premenopausal we anticipated either pregnancy or the onset of menopause in some subjects would limit the ability to ascribe bone changes to HIV/ART rather than these life stage events during the duration of follow up. To prevent this, prior to enrolment, each woman was asked if she was actively planning a pregnancy in the next 12 months and if yes, she was excluded. We also tried to ensure that subjects were having regular menses. Attempts to minimise attrition included providing transportation costs and provision of a meal after visit attendance; transportation costs have been identified as a common cause of losses to follow up (Maskew *et al*, 2007). Public transport around Soweto is limited; most people travel by privately operated 'taxi' minibuses that carry multiple passengers at relatively low cost. Permission from the Ethics Committee was granted to recruit up to 10 further subjects per group to account for potential attrition or loss to follow-up.

In January – February 2011, before the study commenced, an internal 'run through' of the protocol procedures took place in order to identify logistical bottlenecks and areas for improved volunteer flow. As a result, minor changes were made to the study protocol prior to the commencement of the study proper.

## 3.6 Study protocol

**Baseline** evaluation took place on day 0 and involved (Table 3-3):

1. Fasting blood sampling;
2. Fasting urine sampling;
3. Dietary evaluation;
4. Questionnaires: Physical activity, sunlight exposure, and medical/clinical history;
5. Anthropometry, blood pressure (BP), and grip strength;
6. DXA (whole body, lumbar spine, hip, and vertebral morphometry);
7. pQCT (radius and tibia).

Whilst the aim was to complete all the evaluation within one day, the extensive nature meant that this sometimes extended. Most subjects completed the evaluation in one



day, although several who had forgotten to fast were asked to re-attend to provide fasting blood and urine samples on a day close to the first day.

Those in groups Ppres and Plow had immunological measures ($CD_4$ count, and HIV viral loads if available) measured at their normal clinical appointments and the most recent results were collected from patient-held records and used in the analysis. Those in Nref were asked to repeat HIV antibody testing if their negative result was >3 month prior to study entry.

**Table 3-3 Summary of investigations at each study visit**

| Time point months / Investigation | 0 | 6 | 12## |
|---|---|---|---|
| DXA whole body less head<br>• Bone, fat mass and lean mass | √ | √ | √ |
| DXA regional<br>• Lumbar spine<br>• Hip | √ | × | √ |
| DXA vertebral morphometry# | √ | × | √ |
| pQCT#**<br>• Radius<br>• Tibia | √ | × | √ |
| Fasting blood and urine samples<br>• Fasting lipid profile & glucose#~ | √ | √ | √ |
| Anthropometry<br>• BP#<br>• Grip strength# | √ | √ | √ |
| Questionnaires: | | | |
| • Medical/clinical history | √ | √ | √ |
| • Physical activity# | √ | √ | √ |
| • Dietary#* | √ | √ | √ |
| • Sunshine exposure# | √ | √ | √ |
| • Socio economic status measures | × | √ | × |

*Dietary data were collected at each time point and are forming part of an add-on study with Dr Alison Feeley, Stephanie Wrottesely and others.
**pQCT data were collected at 2 time points and are forming part of an add-on study with Dr Kate Ward and others.
~ Add-on study with Dr Shane Norris
# data not presented in this thesis
## This visit constituted the final visit for the PhD component of the study

The 6- and 12-month study visit took place as close to the time as possible with some built in flexibility in the protocol (± 6 weeks) to allow participants to be seen at convenience to them (see Table 3-3).

At 6 and 12 months Nref were offered, and underwent, voluntary repeat HIV-testing to detect incident HIV infection. Ppres and Plow subjects had their $CD_4$ count and HIV viral loads recorded from their patient-held records (if available) as they were measured at their usual clinical facilities. The most recent result was used for the midpoint data.

### 3.7 Recruitment

All subjects were assessed for eligibility and provided informed written consent before enrolment. The study information sheet was written in English; those who were not fluent in English had the information translated into their own language by one of the research staff prior to informed consent being given. Care was taken at the initial screening visit to ensure that potential subjects satisfied the inclusion criteria and did not satisfy any of the exclusion criteria.

Time was taken to explain to participants, and their families if appropriate, the purpose of the study and the safety of unfamiliar technologies such as DXA scanning. This was, in part, to counteract several misconceptions about the nature of biomedical research. Some participants believed that the blood samples would be sold for nefarious purposes and one participant was under the impression that DXA scanning involved the sampling of bone tissue. These concerns could generally be dealt with by careful explanation of the rationale behind the study and various scanning technologies. Many participants had no experience of medical research and great pains were taken to ensure that their consent was fully informed prior to enrolment in the study.

The vast majority of HIV-negative and HIV-positive subjects were recruited via the ZAZI voluntary counselling and testing (VCT) clinic within the Perinatal HIV Research Unit (PHRU) at the Chris Hani Baragwanath Hospital. ZAZI is an open access, free,



confidential VCT service provided by PHRU (http://www.phru.co.za/zazi-vct-centre). Patients attend the clinic, are registered, and undergo pre- and post-test counselling for HIV infection. HIV status is ascertained using a standard, validated point of care test (POCT), the results of which are available in the clinic within approximately 30 minutes, obviating the need for return clinic visits for results. Those who test HIV-positive automatically have further blood samples taken for the measurement of $CD_4$ count. HIV-positive patients are instructed to attend for their $CD_4$ count result after 7 days, at which point a decision is made about any referral for ART initiation, lifestyle advice, and further counselling if required. ZAZI is run by an experienced social worker and staffed by a team of nurses and counsellors.

I undertook a series of formal and informal training exercises with the ZAZI staff explaining the purposes of the study, the inclusion criteria, and the potential benefits to participants. These training sessions involved a PowerPoint presentation, question and answer sessions, and small group discussions.

To advertise the study, A4 size posters were posted in the waiting room of the clinic and more widely around the hospital site alerting patients to what was called 'The Women's Bone Study' (WBS). In addition, ZAZI staff informed their patients of the WBS study pending the result of their HIV-test result. If a patient expressed interest they were given a written information sheet and told that they would be telephoned by a member of the WBS study staff to discuss the study further and, if appropriate, to arrange a visit to the DPHRU office for informed consent and enrolment into the study.

Those who tested HIV-negative at the VCT clinic were able to be screened for the study as soon as they received their HIV-test result. Those who tested HIV-positive had to await the results of the $CD_4$ count to ascertain their eligibility for Ppres or Plow groups.

There is a strong history of biomedical research in Soweto where HIV-negative individuals, in particular, are sought after by research groups for HIV-prevention studies, and retention in WBS was clearly affected (see results: Table 6-2 and Table 6-10).

Recruitment for WBS was initially complicated by a large US-funded vaginal, topical microbicide trial recruiting HIV-negative women from the ZAZI clinic at the same time. Initially, WBS was competing with this HIV-prevention study for HIV-negative volunteers. Furthermore as the microbicide gel may have contained tenofovir (or placebo) and undergone systemic absorption there was a potential effect of this gel on bone. However, the overall numbers of women using the VCT clinic, as well as the reluctance of many to take part in a study that required insertion of a vaginal gel, meant that the Nref recruitment targets were met. Another complication that affected Ppres women was the closure of the 'Wellness' clinic at the PHRU, which saw patients with preserved $CD_4$ counts who had not yet commenced ART. USAID funding was withdrawn from this service in September 2010 and the several hundred patients attending the clinic were redirected to their local community clinics for ongoing clinical care. The Wellness clinic maintained a database of patient contact telephone numbers, however, and eligible patients were contacted by the Wellness staff and asked if they would be willing to consider taking part in WBS. So although this initially threatened to affect recruitment, the target number was reached well within the allotted period.

There was, finally, an unanticipated difficultly recruiting Plow patients. Since there is such a high prevalence of HIV infection in Soweto, any difficulty in recruiting patients requiring ART, prior to initiation of therapy was not anticipated. However, since many patients in this community do not attend for medical assistance until they have advanced disease with pronounced symptoms, it was much more difficult to find patients with low $CD_4$ counts, eligible for ART but who were not yet acutely unwell. We had anticipated recruiting the majority of Plow patients from the adult HIV clinic at Baragwanath Hospital; however patients referred to this service were generally too unwell to be considered for the study since ART initiation could not be delayed whilst baseline evaluations for WBS were being conducted. The problem was overcome, however, by seeking permission from the Gauteng Department of Health to recruit HIV-positive patients from local, community clinics in Soweto. Patients attending these clinics tended



to have less advanced disease and were, therefore, eligible to take part in the study. Sensitisation work along with a multilingual research assistant was carried out with clinical staff at community clinics to inform them of the study and to encourage them to inform their patients. Information sessions were also offered to the patient support groups. However, as a result, Plow subject recruitment was completed approximately 4 weeks later than the other two groups. Plow therefore had 25 (OH)D measurements later than the other groups. This is important since it may have an effect on vitamin D status.

Once women expressed an interest in taking part and were provisionally found to meet the inclusion criteria via telephone interview, they were invited to the DPHRU research offices to undergo a formal screening process by a clinician and enrolment if appropriate. Enrolment was considered complete after the subject had completed the baseline evaluation, described in detail in Chapter 4. As the Chris Hani Baragwanath Hospital is spread over a very large area most women were met at the ZAZI clinic, by a research assistant with whom they were familiar, who accompanied them to the DPHRU building.

Each participant was reimbursed ZAR 50.00 (≈£3.50) per study visit, in line with local, South African Ethics Committee standards. Since participants had to arrive fasted they were also given refreshments, (tea, coffee or fruit juice, and cheese and salad sandwiches), following collection of blood and urine samples. At each study visit, participants also had their BP measured, finger prick glucose testing, and had their BMI calculated. If any of these tests were abnormal by South African standards the participant was referred for further assessment to the relevant health care professional (4.10). Given that access to any type of healthcare service is difficult in poor communities in South Africa, this type of opportunistic screening was viewed in a very positive light by participants.

Prior to each study visit, participants were contacted by telephone to remind them of their appointment. Attempts were made to maximise retention by ensuring that each

study visit did not take too long to complete. Since it is common for mobile telephone numbers to be changed frequently in South Africa, not only were participants' mobile telephone numbers collected but also the names and contact numbers of next of kin and of two other friends or family. As a result it was possible to contact participants unavailable on their own contact number. Physical addresses were recorded wherever possible, although this was difficult for more informal settlements because street and building numbers are not always readily available.

All participants were provided with the DPHRU toll-free number so that they could call the office without cost. Appointments were made up to 2 weeks in advance; participants were then called the day before their appointment as a reminder. A specific WBS mobile phone was also used to send a text message the evening before each study visit as another form of reminder. This message also reminded the participant to fast overnight. Mobile phone usage in urban South Africa is extremely high and virtually all participants had access to a mobile telephone. However, even with these intensive attempts to encourage attendance, it was very common for women to fail to attend on the appointed day, so visits often had to be rescheduled three or more times. Letters provided to employers explaining that a participants was absent from work because of participation in a study often succeeded in allowing participants to take time off work without being penalised.

Every effort was made to make the experience of each study visit as pleasant as possible. For many poor black South Africans their experience of a health care facility involves several hours of waiting in crowded circumstances in order to be seen by a clinician. Patients often have to give up an entire day to be seen at a clinic or in hospital. The experience can be very stressful and is perceived to lack privacy and care. Whilst I made it clear to participants that they were attending a research and not a clinical facility, they clearly appreciated the professional and courteous manner in which they were treated by various members of the study staff. This is likely to have increased retention in the study. Each participant was offered a written copy of her relevant study



visit results (BP, glucose, weight, and BMI) and this was also generally very well received.

# 4 Methods

## 4.1 Selection of methods

The following methods were chosen to try and best answer the specific research questions outlined in the aims and objectives.

Anthropometry was used to measure height, weight, and waist and hip circumferences. To compare baseline aBMD and body composition the method of choice was DXA, which allows for measurement of bone mineral content, area and density. It also allows for estimation of body fat and fat free mass. Laboratory analysis of blood and urine allowed for measurement of 25(OH)D and biochemical markers of bone metabolism. Questionnaires allowed for assessment of medical/clinical history, socio-economic status (SES), physical activity, sunshine exposure, and dietary intake. pQCT measurements of tibia and radius were conducted as part of an add-on study, separate from my PhD research.

## 4.2 Study team and responsibilities

The team who were involved in data collection and sample processing were as shown in Table 4-1.

**Table 4-1 Study staff in South Africa**

| Individual | Role | Duties |
|---|---|---|
| Matthew Hamill# | Principal investigator | • Study physician<br>• Clinical oversight<br>• Consent<br>• Phlebotomy<br>• Fasting blood glucose<br>• HIV-antibody testing<br>• Medical/clinical history (Appendix 2), Physical activity, socioeconomic status questionnaires |
| Dimakatso Mafokwane* | Research assistant | • Consent<br>• Phlebotomy<br>• Fasting blood glucose<br>• DXA, pQCT<br>• Telephone appointments |
| Mary Mokhale | Research nurse | • Phlebotomy<br>• Fasting blood glucose |
| Thokozile Lephoto | Research sister | • Consent<br>• Phlebotomy<br>• Fasting blood glucose<br>• DXA, pQCT |
| Eliza Tsoeu** | Research assistant | • DXA, pQCT |
| Thabile Sibiya | Research assistant | • DXA, pQCT |
| Mantoa Langa | Research assistant | • Food Frequency Questionnaire (baseline)<br>• Telephone appointments |
| Meike Hlalele*** | Research assistant | • Anthropometry<br>• Dietary questionnaires (6 and 12 months) |
| Nomses Baloyi | Research assistant | • All questionnaires<br>• Anthropometry |
| Zandile Lephoto | Research assistant | • All questionnaires |
| Mampho Ratshihule | Domestic worker | • Food and beverage preparation |
| Usanda Puta | Laboratory technician | • Laboratory analysis |
| Machuene Poopedi | Laboratory technician | • Laboratory analysis |
| Jackson Mabasa | Research assistant | • Biological sample processing and storage |
| Alison Feeley | Post-doctoral scientist | • Dietary analysis |
| Stephanie Wrottesley | Research assistant | • Dietary analysis |

*Was a member of the study team until August 2011
#carried out >90% of consent and phlebotomy
**Carried out >90% of DXA/pQCT
***Carried out >90% of anthropometry measures

**Figure 4-1 Some of the study team members in South Africa**

Left to right: Nomses Baloyi, Mantoa Langa, Zandile Lephoto, Dimakatso Mafokwane, Eliza Tsoeu, Mampho Ratshihule, Thokozile Lephoto, Meike Hlalele

All the procedures described below were conducted in accordance with DPHRU operating procedures.

## 4.3 Anthropometry

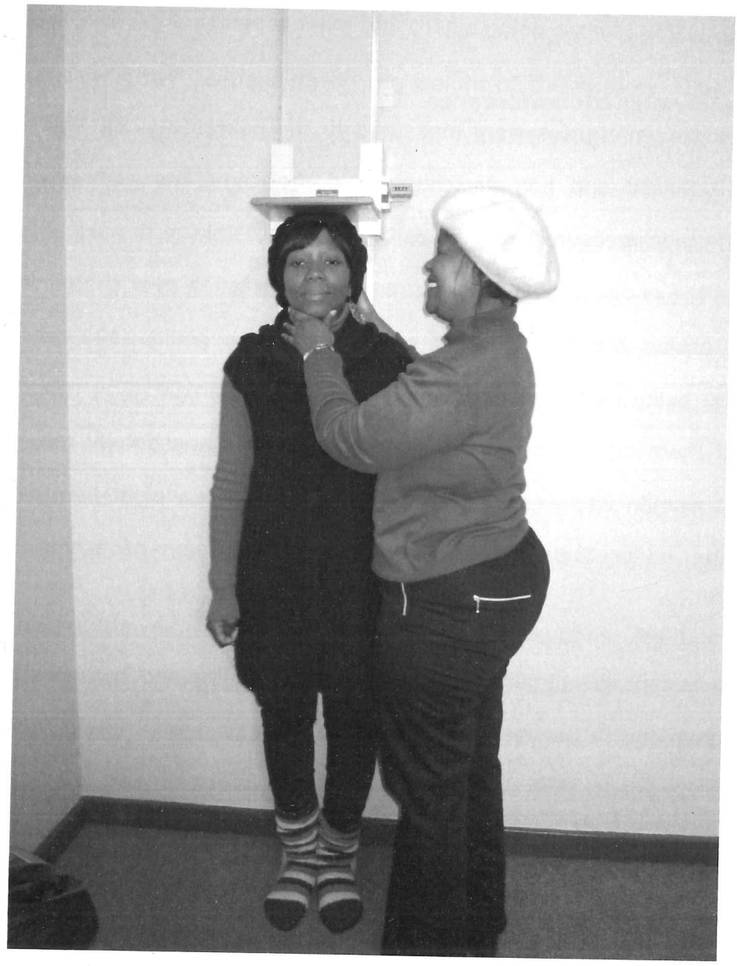
Anthropometry was undertaken by a trained, female research assistant who carried out the measurements in the vast majority (>90%) of subjects. These measures were undertaken in a room used exclusively for anthropometric measurements to ensure that the participants had privacy and to ensure that the equipment used was not tampered with. The DXA whole body scan was used to provide measures of fat and lean mass and to estimate percentage (%) body fat (see Section 2.2.7.1).

### 4.3.1 Height

Height was measured to the nearest 0.1 cm using a permanent wall-mounted stadiometer (Holtain, Crosswell, UK) (see Figure 4-2). Subjects were asked to remove footwear and to stand erect with the posterior aspect of the heels and shoulders against the wall, keeping knees and back straight with the Frankfort plane (i.e. the line between the left eye and superior border of the external auditory meatus) in the horizontal position. In subjects who wore irremovable, sewn-in wigs or hair weaves the moveable portion of the hair piece was flattened and compressed as close to the skull as possible without causing the subject discomfort. Where buttock adiposity prevented the subject from holding the shoulders against the wall without standing upright, she was asked to stand as close to the wall as was feasible.



Figure 4-2 Volunteer having her height measured



### 4.3.2 Weight

Weight was measured to the nearest 0.1kg using an electronic digital scale (Tanita, TBF-410 MA Body Composition Analyzer, Tanita Corporation of America, Inc., Illinois, USA) with participants wearing light clothing and no shoes.

### 4.3.3 BMI

Body Mass Index (BMI) was calculated as the participant's weight in kilograms divided by the square of their height in metres ($kg/m^2$). Underweight, normal, overweight, and obese were defined as BMI <18.5, 18.5-24.9, 25-29.9, $\geq$30.0 respectively (WHO, 2006).

A BMI of >25 or <18.5 triggered a referral onwards to the dietetic clinic at Chris Hani Baragwanath Hospital for on-going advice and management.

#### 4.3.4 Hip and waist circumferences

Hip and waist circumferences were measured to the nearest 0.1cm. The tape measure used was plasticised and non-stretchable and the same measure was used for all subjects. The tape measure was held as tightly as possible to the skin without causing depression of the underlying skin. Measurements were taken over the minimum amount of clothing; asking subjects to expose their abdomen could have been considered intrusive. Any bulky item was removed prior to measurement. The research assistant ensured that there was no concealed padding as it was anecdotally reported common practice that women who consider themselves to be underweight use towels or other fabric padding under their clothes to give the impression of accentuated buttock contours.

**Waist** measurement was obtained with the subject standing and in a relaxed pose at the minimum circumference between the bottom of the rib cage and the tip of the iliac crest. Where adiposity made this difficult to visualise, the torso was gently palpated to ascertain the area of minimum circumference.

**Hip** measurement was taken at the maximum circumference between the waist and top of the thighs.

**Waist: Hip** ratio was calculated by dividing waist by hip circumferences ($W_{circ}/H_{circ}$).

### 4.4 Assessment of bone health

#### 4.4.1 Dual-energy X-ray absorptiometry (DXA)

DXA was performed using an Hologic QDR 4500A dual-energy X-ray absorptiometer (DXA) (Model: Discovery W (S/N 71201) software version 12.5:7 Hologic, Inc., Waltham, MA, USA) according to standard procedures, see Section 2.2.7. This DXA machine provided measurement of the following sites: whole body (WB), lumbar spine (LS), total hip (TH) and femoral neck (FN). Manufacturer's instructions were followed for correct positioning for hip, lumbar spine, and WB scans. Scans were conducted with subjects



wearing light clothing having removed metal objects such as jewellery, belts and clothes containing metal. Scans were performed using the automatic scan mode, i.e. 'array', 'fast array' or 'slow array', depending on the weight of the subject. DXA was used to measure BMC (g), BA ($cm^2$) and aBMD ($g/cm^2$) of WB, TH, FN and LS (L1-L4). A full set of DXA scans was obtained at both baseline and 12 months, only a WB scan was carried out at the six month visit. The reason for this was that it was felt to be unlikely that changes in TH, FN, and LS would be detectable at six months. DXA measurements of WB were analysed using the 'WB-less head' mode (WBLH) as many women wore wigs and hair weaves that could not be removed prior to scanning. This artificial hair was of similar density to soft tissue and therefore would affect DXA measurements of the head region. Total fat and lean body mass (g) were also measured by DXA. aBMD SD-scores (SDS) were derived using the HIV-negative controls as the reference population.

Based on a set of the manufacturer's unpublished assumptions, the bone edges are automatically delineated from soft tissue using the attenuation profiles generated. The Hologic software (Hologic QDR 4500A (Model: Discovery W (S/N 71201) software version 12.5:7 Hologic, Inc., Waltham, MA, USA) defines BA, and BMC and aBMD are measured within this area. This is a fanbeam scanner that uses a broad X-ray beam and multiple detectors to allow the measurement of attenuation in a row of pixels simultaneously.

Radiation doses used in DXA are low and deemed to be safe. They represent little over normal background radiation doses (UNSCEAR, 2000; NESCA, 2009; Radiological Society of North America Inc. (RSNA), 2010). A set of scans was carried out three times during the 12-month study period, calculated to be 0.0202 mSv (Radiological Society of North America Inc. (RSNA), 2010), this compares with 0.04 mSv for a chest radiograph (Table 2-2). The global annual, background radiation is measured at 2.4 mSv and South Africa's average is close to this although Johannesburg's is likely to exceed this amount due to the presence of gold mining activity and associated uranium deposits in surface soil (personal communication, James Larkin). Therefore the DXA radiation dose for each set

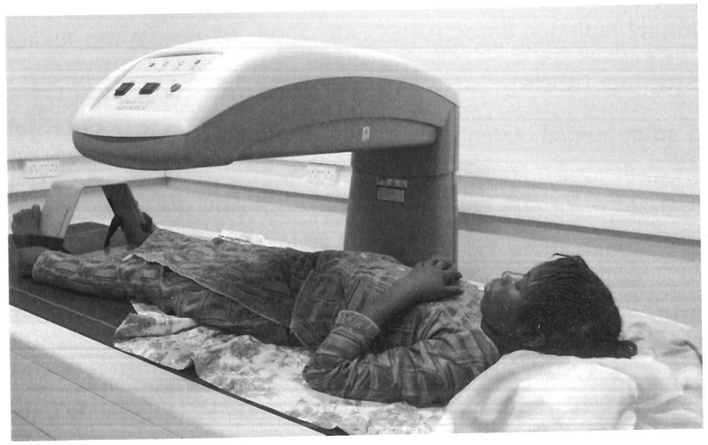
of scans was equivalent to approximately one day's background radiation dose and for the whole set much less than annual background exposure.

Prior to scanning, the subject's biographical details were checked to ensure the correct individual was being scanned, and name, date of birth, weight, and height were entered. Prior to each DXA scan, the woman was asked if she might be pregnant and a pregnancy test performed; the scan was only conducted if the pregnancy test was negative (see Section 3.4).

#### 4.4.1.1 The hip scan

The left hip scan was acquired with the subject lying on her back and with foot positioner between her ankles (see Figure 4-3). This was then aligned with the subject's midline. The hip was internally rotated $25^0$ and the medial edge of the foot placed against the triangle of the foot positioner with the foot held in plantarflexion. The foot was secured on the foot brace with a strap. The femur was aligned to be parallel to the table edge to provide adequate rotation to ensure correct positioning of the neck box for analysis. The leg was then abducted from the midline in order to straighten the femur. The scanner was positioned with the laser light guide positioned over the middle of the left thigh, approximately 7 cm below the level of the greater trochanter. The machine then automatically carried out the measurement.

**Figure 4-3 A participant undergoing a hip DXA scan**



Reproduced with permission MRC Keneba. Used to illustrate positioning for a TH DXA

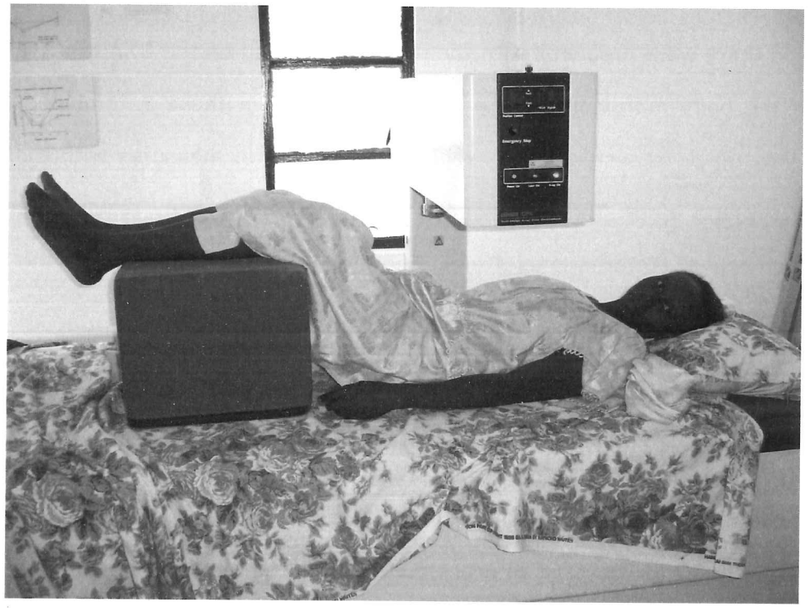


#### 4.4.1.2 The lumbar spine scan

The lumbar spine scan was acquired by laying the subject on her back with the body positioned so that the spine was straight on the table mattress and the shoulders at the upper scan limit line (see Figure 4-4). The pelvis and shoulders were aligned in a straight line and centred on the marks on the table pad. The knee positioner was then placed under the subject's legs and positioned until the femurs were parallel with the edge of the block creating a 45 - $90^0$ angle at the knees, reducing the lumbar lordosis and so reducing projection errors in the scan. Finally the subject was instructed to place her arms above her head or by her sides, whichever was the most comfortable, and asked to breathe normally while remaining still.

Vertebral assessment via lateral DXA was carried out to determine vertebral fracture and/or deformity prevalence within this cohort and to serve as a baseline on which to investigate incident osteoporotic fracture in the future.

**Figure 4-4 A participant undergoing a lumbar spine DXA scan**



Reproduced with permission MRC Keneba. Used to illustrate positioning for a LS DXA

#### 4.4.1.3 The whole body scan

This scan was obtained with the subject lying on her back and instructed to keep looking at the ceiling while remaining still (see Figure 4-5). The body was straight on the table using the centre lines at the head and feet ends of the table as a gauge. The body was positioned within the scan limit area whenever possible. Arms were placed by the sides with arms pronated and separated from the thighs; the feet were held in plantarflexion and held together using a fabric tie.

The high rates of adiposity in the study participants meant that a large number of subjects were unable to fit within the scan field. For those individuals who were too wide to fit all of their body into the scan field for the WB scan, initially the individual was positioned slightly to the right to try and fit in all of the body except the most lateral aspect of the right arm. As a result a number of subjects were scanned so that a portion of their right arm was not within the scan field and a correction applied based on left arm values (Micklesfield *et al*, 2007). The main reason was that gluteal adiposity caused the arms to be splayed away from the body (Figure 4-6). During the baseline data collection time period there were problems with the mattress slipping on the DXA table resulting in a shift of the body marginally to the right. As a result of these 2 combined factors a decision was made to systematically replace the right arm measures with those of the left in all subjects' data. This technique is known as "whole body result by imputation from shifted scans" (International Atomic Energy Agency, 2010) and used the following equation (in this example for BMC):

$$BMC_{total\ imputed} = BMC_{total\ shifted} - BMC_{right\ arm} + BMC_{left\ arm}$$ (modified from (International Atomic Energy Agency, 2010)

This process was applied also for BA, and aBMD was recalculated using the new BMC and BA values. In order to compare all scans consistently the method was continued for the 6 and 12 months scans (see Figure 4-7).



**Figure 4-5 Volunteer undergoing whole body DXA**

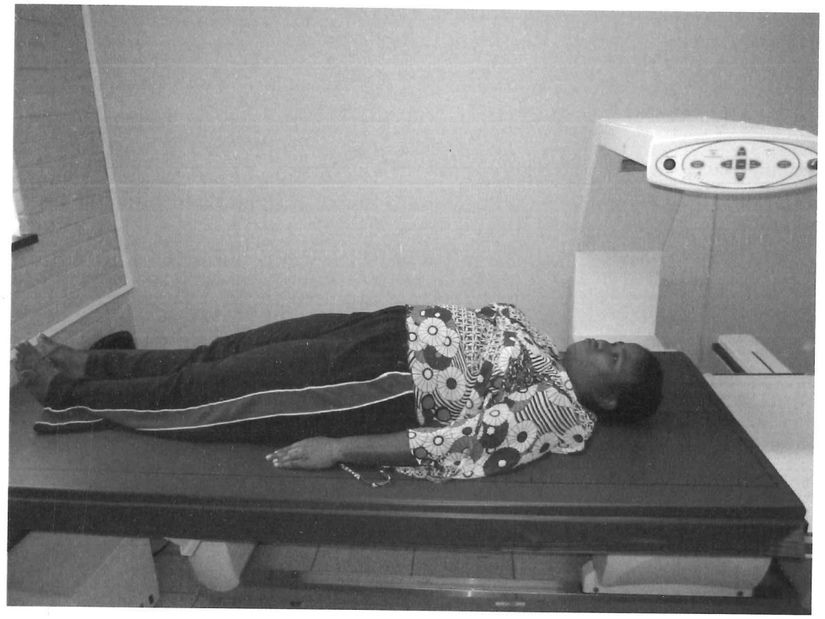


Figure 4-6 Example of right sided 'over-positioning' for WB DXA scan

**BIRTH TO TWENTY YOUTH MATURITY**
**BARA SITE**

| | | |
|---|---|---|
| Name:<br>Patient ID: WBS 142B<br>DOB: 31 October 1966 | Sex: Female<br>Ethnicity: Black | Height: 155.9 cm<br>Weight: 109.2 kg<br>Age: 44 |

Referring Physician: DR.MATTHEW HAMILL

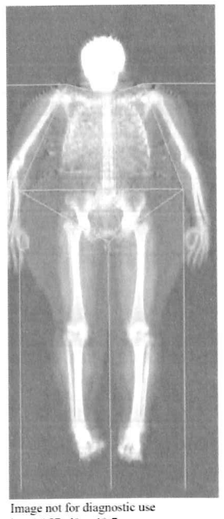
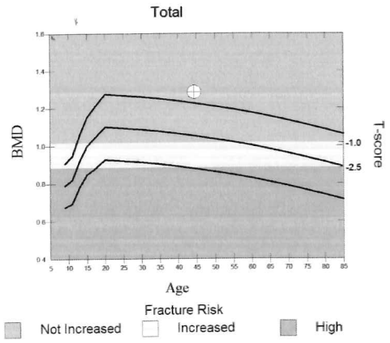


Image not for diagnostic use
k = 1.157, d0 = 40.7
318 x 150




T-score vs. White Female; Z-score vs. White Female. Source:Hologic

**Scan Information:**

Scan Date: 22 March 2011 ID: A03221105
Scan Type: a Whole Body
Analysis: 28 October 2011 08:51 Version 12.5:7
Auto Whole Body
Operator: ET
Model: Discovery W (S/N 71201)
Comment:

**DXA Results Summary:**

| Region | Area (cm²) | BMC (g) | BMD (g/cm²) | T-score | PR (%) | Z-score | AM (%) |
|---|---|---|---|---|---|---|---|
| L Arm | 217.73 | 187.92 | 0.863 | | | | |
| R Arm | 197.05 | 178.52 | 0.906 | | | | |
| L Ribs | 139.69 | 117.81 | 0.843 | | | | |
| R Ribs | 192.76 | 160.18 | 0.831 | | | | |
| T Spine | 87.41 | 107.32 | 1.228 | | | | |
| L Spine | 46.04 | 53.85 | 1.169 | | | | |
| Pelvis | 168.57 | 234.72 | 1.392 | | | | |
| L Leg | 411.28 | 509.74 | 1.239 | | | | |
| R Leg | 412.45 | 494.99 | 1.200 | | | | |
| Subtotal | 1872.99 | 2045.04 | 1.092 | | | | |
| Head | 229.83 | 657.83 | 2.862 | | | | |
| **Total** | **2102.82** | **2702.88** | **1.285** | **2.1** | **117** | **2.7** | **122** |

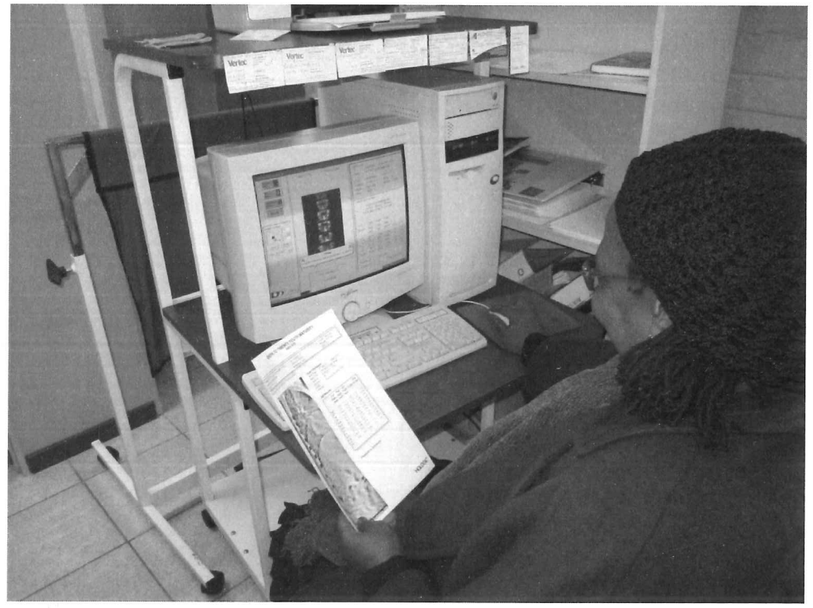
Total BMD CV 1.0%, ACF = 1.029, BCF = 0.954

**Physician's Comment:**

HOLOGIC



Figure 4-7 DXA operator at DPHRU



(Eliza Tsoeu)

### 4.4.2 Quality assurance (QA)

Throughout the course of the study daily quality checks were conducted using spine phantoms to test scanner performance and are demonstrated in Figure 4-8, Figure 4-9, and Figure 4-10.

Typically, the coefficients of variation for measurements of the manufacturer's phantom were 0.39%, 0.34%, and 0.23% for bone mineral content (BMC), bone area (BA) and bone mineral density (BMD) respectively. The coefficients of variation for the DXA operator (2 repeat measures on 10 volunteers) of the lumbar spine and proximal femur BMD were 0.65% and 0.97% respectively (personal communication, Prof Shane Norris).

Figure 4-8 Lumbar spine BMC QA

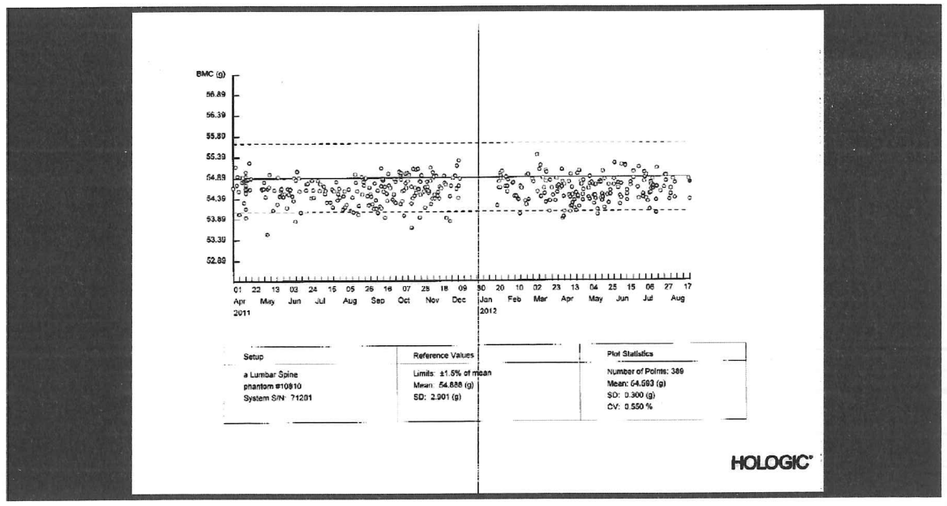



Figure 4-9 Lumbar spine BA QA

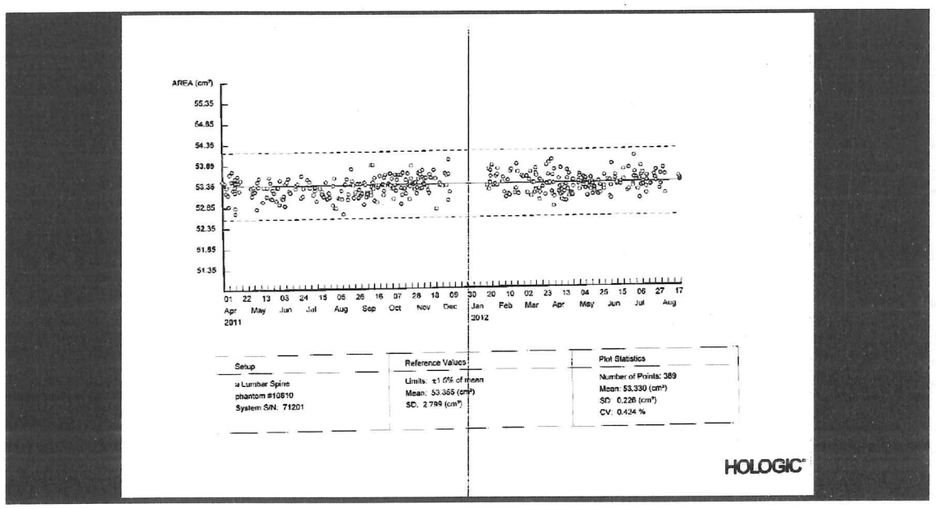
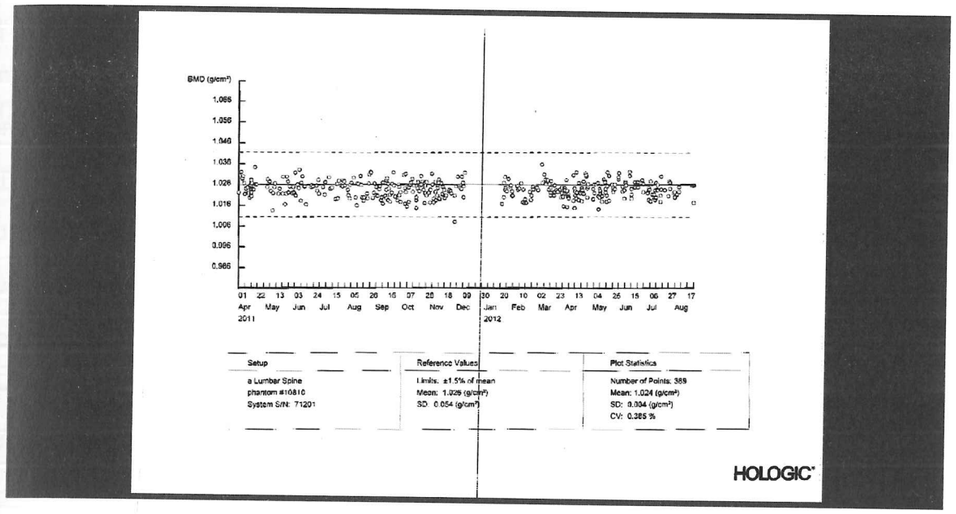





Figure 4-10 Lumbar spine BMD QA




### 4.4.3 Scan quality

All scans were assessed by me after training by an experienced senior research scientist at MRC HNR (Dr Kate Ward). The scans were reviewed and graded each day and where possible scans that were technically poor were repeated.

**Scan acquisition:** Post-analysis, each scan was graded from one to three, as is standard practice in Nutrition and Bone Health (NBH) MRC HNR, according to the ability to extract valid data from the scan, taking into account: the positioning of the subject and consequently the ability to correctly position the regions of interest; artefacts in the scan caused by clothing and jewellery or the presence of metallic joint material. Grade three scans were those in which one or more of the above considerations were deemed to have affected the scan quality and the ability to generate valid data. Grade two scans were those in which one or more of the considerations was in effect but where the effect was judged less likely to affect the resultant data. Grade one scans were those unaffected by the above considerations. A small number of spine scans were excluded from data analysis due to severe problems preventing the scans from being analysed, such as visible evidence of soil in the gut lumen due to geophagy. Scans graded as a three were repeated whenever practicable.

**Scan analysis:** After analysis, each scan was graded from one to three, as is standard practice in NBH, according to the clarity of the scan image, based on the ability of the person grading and the analysis program to correctly identify bone edges in the scan. Reductions in image clarity occur as a result of subject movement during the scan and lead to a reduced confidence in the data extracted. Scans graded as three were those in which movement by the subject was deemed to have substantially affected the ability to extract valid data. Grade two scans were those in which movement was discernible but not considered extensive enough to affect data validity. Grade one scans were those where little or no movement was evident. Other reasons to grade a scan as a grade two would include the inability to completely separate arm from torso on WB scan due to overlying fat or the inclusion of T12 rather than L1 on LS scan. Grade 2 scans would be reanalysed by the DXA technician (ET) in order to improve the detection of the correct bone edge whenever feasible. They were then re-evaluated by me as the Principal Investigator.

Whilst the grading of scans was subjective it was carried out by the same investigator and was therefore consistent. If a scan was of uncertain quality it was discussed with Professor John Pettifor and a decision made about its quality and whether it was suitable to be included in the analysis.

## 4.5 Collection of biological samples

### 4.5.1 Fasted blood collection

Blood was collected in the vast majority of cases by me (see Section 4.2) using standard procedures with the subject sitting in a comfortable position on a couch. A suitable vein was identified using inspection and palpation, the skin cleaned using an alcohol swab, allowed to dry and then blood drawn using the Becton Dickinson vacutainer system and blood collection tubes (BD, Plymouth UK). After the needle was withdrawn the area was dressed with a cotton wool pad and sticking plaster. The reason to measure fasting samples is to avoid any circadian or post-prandial variation in serum concentration. All laboratory measurements were carried out by two trained laboratory technicians (see



Section 4.2) in the on-site DPHRU laboratory. The samples obtained are shown in Table 4-2.

**Table 4-2 Blood samples**

| | | WBS analysis | Planned later analysis* | How sample processed | Samples storage |
|---|---|---|---|---|---|
| Serum | Plain CAT tubes (3 x 10 ml) | 25(OH)D<br>Routine chemistry:<br>- Calcium<br>- Phosphate<br>- Albumin<br>Creatinine<br>- ALP<br>(see section 5.8) | CTx<br>Osteocalcin<br>BALP<br>FSH<br>Lipid profile | Allowed to clot at room temperature for 20-60 minutes<br>Centrifuged within 3 hours | -20 $^{oC}$ (4 weeks), thereafter -80$^{oC}$ in 3ml polypropylene storage tubes |
| Plasma | EDTA K2E 7.2 mg tubes (4 ml) | | PTH | On ice immediately<br>Spun within 3 hours<br>Centrifuged within 3 hours | -20 $^{oC}$ (4 weeks), thereafter -80$^{oC}$ in 3ml polypropylene storage tubes |
| Plasma | FX 10mcg, 8 mcg tubes (4 ml) | | Glucose<br>Lactate | On ice immediately<br>Centrifuged within 3 hours | -20 $^{oC}$ (4 weeks), thereafter -80$^{oC}$ in 3ml polypropylene storage tubes |

*Beyond the scope of this thesis

#### 4.5.1.1 Fasting blood glucose

A finger prick blood glucose test was undertaken at each study visit. There were two reasons for this. Firstly to alert the study team to previously undiagnosed diabetes mellitus and secondly, to confirm that the subject was likely to have undertaken an overnight fast. The use of finger prick glucose monitoring has historically been used for research studies at Bt20/DPHRU to provide evidence of appropriate fasting state. A value of ≤ 7.0 mmol/l was considered consistent with an overnight fast. Finger prick blood sampling was obtained following operators instructions using the Roche, Accutrend® GCT (153762) (Roche, Basel, Switzerland). A blood glucose reading of >11.1 mmol/l triggered an immediate referral onwards to the subject's local clinic facility for checking and treatment as necessary. A result between 7.0-11.0 mmol/l triggered a routine referral to the subject's local clinical facility for repeat testing and appropriate management.

### 4.5.2 Fasted urine collection

Urine was collected after an overnight fast, though water was permitted to be drunk. The first urine of the day was voided and the second was collected into a sterile universal container (4.7.1). The spot urine sample was collected just before or immediately after

phlebotomy. Approximately 20 – 30 ml of urine were collected into a sterile universal container and divided into 2 samples in the onsite DPHRU laboratory. 5 ml of urine was acidified with 1 drop (=50µl) of concentrated (38%) hydrochloric acid (HCl). Another 5 ml of unacidified urine was stored for future analysis. The samples obtained are shown in Table 4-3.

**Table 4-3 Urine samples**

| | WBS analysis | Planned later analysis* | How sample processed | Samples storage |
|---|---|---|---|---|
| Universal sterile container | Urinary βHCG | RBP Protein: Creatinine ratio | Refrigerated within 3 hours then stored | -20 $^{oC}$ (4 weeks), thereafter -80$^{oC}$ in 5ml polypropylene storage tubes |
| Universal sterile container | Creatinine Calcium Phosphate (see Section 4.7) | | Acidified and refrigerated within 3 hours then stored | -20 $^{oC}$ (4 weeks), thereafter -80$^{oC}$ in 5ml polypropylene storage tubes |

*Beyond the scope of this thesis

### 4.5.3 HIV-antibody testing

At the 6-month and 12-month visits, Nref subjects were offered repeat HIV antibody testing if they had not already undergone repeat testing at a local community facility within 6 weeks prior to the visit. HIV-testing used the Alere Determine™ rapid HIV-antibody test (Alere San Diego, Inc. San Diego, CA). For convenience this test was taken at the same time as the fasting blood samples but did not require the subject to be in a fasted state. This technique uses point of care technology, the results of which were available in approximately 15 minutes. Additional full verbal consent was obtained prior to the test being carried out. The consent was taken, and result given, by a trained research assistant translating for the Principal Investigator if the subject was unable to communicate well in English. The whole blood sample was obtained by finger prick blood testing and testing algorithms were followed as per manufacturer's instructions. Each test had an internal control to ensure quality control. If the test was reactive then the subject was referred to the ZAZI clinic for confirmatory serological testing and $CD_4$ count. http://www.who.int/diagnostics_laboratory/documents/guidance/determine.pdf).



## 4.6 Laboratory methods (blood)

### 4.6.1 25-hydroxyvitamin D

Blood was collected for 25(OH)D analysis, measured by chemi-luminescent immunoassay (Liason) kit DiaSorin Inc., Stillwater, MN, USA). The blood samples were allowed to clot for a minimum of 20 min at room temperature, and the serum was aliquoted and stored at −20 C until analysed. All samples were run in duplicate. The inter-assay CV for low and higher 25(OH)D controls was 10 and 9 %, respectively, whereas the intra-assay CV was 8 and 6 %, respectively. The DPHRU laboratory participates in the International Vitamin D External Quality Assessment Scheme and holds the certificate of proficiency.

### 4.6.2 Calcium

Serum calcium was corrected for serum albumin using the following formula (Ramrakha *et al*, 2010):

**Corrected calcium = measured calcium + {[40 – serum albumin (g/L)] x 0.02}**

Serum (and urinary (see section 4.7)) calcium was measured using a colorimetric method, whereby the reagent (Arsenazo III) specifically binds to calcium forming a coloured complex measured at 660 nm. The amount of calcium present in the sample is directly proportional to the intensity of the resulting coloured complex. The assay was performed using a Randox Daytona analyser with a 660 nm optical analyser using Randox calibration serum level 3 for calibration. Randox assayed multi-sera, levels 2 and 3 were used for daily quality control, if the values obtained fell outside of the specified range then the analysis was repeated. The normal range specified is 2.02-2.65 mmol/l for serum, the minimum level of detection for calcium has been determined as 0.20 mmol/l.

The following data listed in tabular form in the remainder of this chapter are those provided by information in the manufacturers kit inserts.

The intra-run precision was as follows:

| Serum calcium | Level 1 | Level 2 | Level 3 |
|---|---|---|---|
| Mean (mmol/l) | 1.81 | 2.28 | 4.02 |
| SD | 0.01 | 0.03 | 0.03 |
| CV (%) | 0.88 | 1.51 | 0.83 |
| N | 20 | 20 | 20 |

The inter-run precision was as follows:

| Serum calcium | Level 1 | Level 2 | Level 3 |
|---|---|---|---|
| Mean (mmol/l) | 1.66 | 2.13 | 3.98 |
| SD | 0.07 | 0.06 | 0.09 |
| CV (%) | 4.35 | 2.99 | 2.16 |
| N | 20 | 20 | 20 |

### 4.6.3 Phosphate

Serum phosphate was measured using a Randox UV method whereby the inorganic phosphorus binds with ammonium molybdate in the presence of sulphuric acid forming a phosphomolybdate complex measurable at 340 nm. The assay uses an endpoint method and single point calibration.

The manufacturers determined normal range for serum is 0.87 to 1.45 mmol/l; the minimum detectable concentration of inorganic phosphorus, with an acceptable level of precision, was 0.144 mmol/l.

The intra-assay precision was as follows:

| Serum phosphate | Level 1 | Level 2 | Level 3 |
|---|---|---|---|
| Mean (mmol/l) | 0.492 | 1.20 | 2.18 |
| SD | 0.011 | 0.022 | 0.013 |
| CV (%) | 2.25 | 1.83 | 0.61 |
| N | 20 | 20 | 20 |

The inter-assay precision was as follows:

| Serum phosphate | Level 1 | Level 2 | Level 3 |
|---|---|---|---|
| Mean (mmol/l) | 0.482 | 1.72 | 2.64 |
| SD | 0.017 | 0.027 | 0.031 |
| CV (%) | 3.52 | 1.59 | 1.16 |
| N | 20 | 20 | 20 |



### 4.6.4 Creatinine

Creatinine is the product of breakdown of creatinine and creatinine phosphate in muscle and is a nitrogenous waste product. Creatinine is not further metabolised but excreted in the urine via the kidney. It is produced and excreted in a constant fashion that is proportionate to body muscle mass. Because of its constant excretion characteristics it is used mainly as a proxy for renal function or to correct for urine dilution. The advantages of measuring creatinine as a marker of renal function is that it is relatively independent of protein and water intake, exercise and rate of urine production. Since its rate of production is constant, elevation of serum creatinine is indicative of renal under-excretion although it is limited in situations of extremes of muscle mass and differs between ethnic groups.

Measurement of serum creatinine was by Randox colorimetric method whereby creatinine in alkaline solution reacts with picrate to form a coloured complex; it is the rate of formation of complex that is measured.

The minimum detectable concentration of serum creatinine with an acceptable level of precision was determined as 26µmol/l. The manufacturer's determined normal range was 44 – 80 µmol/l for women.

The within-run precision was as follows:

| Serum creatinine | Level 1 | Level 2 | Level 3 |
|---|---|---|---|
| Mean (µmol/l) | 65.3 | 131.4 | 384.2 |
| SD | 2.64 | 3.38 | 8.35 |
| CV (%) | 4.0 | 2.6 | 2.2 |
| N | 88 | 87 | 88 |

The total-run precision was as follows:

| Serum creatinine | Level 1 | Level 2 | Level 3 |
|---|---|---|---|
| Mean (µmol/l) | 65.3 | 131.4 | 384.2 |
| SD | 3.27 | 4.29 | 14.8 |
| CV (%) | 5.0 | 3.3 | 3.8 |
| N | 88 | 87 | 88 |

Serum creatinine assays for all samples collected at baseline were conducted at the same time with the same kit batch; those collected at the 6 and 12 months timepoints were performed some months later using a different kit batch, which had slightly different quality characteristics. All urine samples from each of the three timepoints were analysed together using the second kit batch. All baseline values for serum creatinine were therefore corrected using the formula x78.55/69.2 in order to ensure consistency across the longitudinal series and in the calculation of TMP/GFR without affecting any observed between-group differences at each timepoint.

### 4.6.5 Estimated glomerular filtration rate

Estimated glomerular filtration rate (eGFR) was calculated using the Modification of Diet in Renal Disease (MDRD) study formula. It estimates the glomerular filtration rate (GFR) using the serum creatinine, age, gender, and ethnicity, i.e.: **eGFR (ml/min/1.73m$^2$) = 175 x ((serum creatinine (µmol/l)/88.4)$^{-1.154}$) x age (years)$^{-0.203}$ x 0.742 if female and x 1.21 if African-American** (Levey *et al*, 2006; Stevens *et al*, 2010).

However, as per South African guidelines, the African-American multiplier was not applied to black African subjects as it tends to lead to an underestimate of eGFR in this population when compared with African-Americans, using plasma clearance of chromium-51-EDTA ((51)Cr-EDTA) as a gold standard method of measuring GFR (van Deventer *et al*, 2008).

### 4.6.6 Albumin

Albumin is the most abundant serum protein accounting for 55 – 65% of total protein. It is hepatically synthesised and has a half-life of 2 – 3 weeks. The principal biological functions of albumin are to maintain water balance in serum and plasma and to transport and store a variety of ligands such as calcium, fatty acids, drugs, and hormones such as thyroxine. Albumin also provides a source of endogenous fatty acids. In catabolic disease states, such as under-nutrition, cancer, and untreated HIV infection, albumin synthesis may fail to keep pace with its metabolism resulting in low serum albumin concentrations.



Serum albumin was measured using the Randox Bromocresol Green assay. This technique is based on albumin's quantitative binding to the indicator 3,3', 5,5'-tetrabromo-mcresol sulphonophthalein (Bromocresol Green (BCG)). The albumin-BCG-complex absorbs maximally at 578 nm.

The minimum detectable concentration of albumin in serum with an acceptable level of precision was determined as <4.7 g/l. The manufacturer's determined normal range for adults is 38-44 g/l.

The within-run precision was as follows:

| Serum albumin | Level 1 | Level 2 | Level 3 |
|---|---|---|---|
| Mean (g/l) | 14.6 | 35.6 | 43.1 |
| SD | 0.24 | 0.43 | 0.53 |
| CV (%) | 1.62 | 1.20 | 1.23 |
| N | 20 | 19 | 20 |

The between-run precision was as follows:

| Serum albumin | Level 1 | Level 2 | Level 3 |
|---|---|---|---|
| Mean (g/l) | 14.0 | 30.6 | 45.4 |
| SD | 0.49 | 0.67 | 0.79 |
| CV (%) | 3.48 | 2.19 | 1.74 |
| N | 20 | 20 | 20 |

### 4.6.7 Alkaline Phosphatase (ALP)

Measurements of alkaline phosphatase (ALP) activity are used in the diagnosis and investigation of hepatobilary disease and bone disease associated with increased osteoblastic activity. Total ALP activity cannot distinguish between that produced by bone and liver, however, it remains a useful adjuvant in assessing bone health and bone formation. Bone ALP (BALP) is the bone-specific isoenzyme that can identify ALP liberated from osteoblasts and is therefore an important biomarker of bone formation.

Serum ALP was measured using the Randox optimised standard colorimetric method at 37$^0$C. The substrate p-nitrophenyl is hydrolysed by alkaline phosphatase from the sample, in the presence of magnesium ions, to form p-nitrophenol, which is yellow in

colour and can be read at 405 nm. The intensity of colour produced is proportional to the alkaline phosphatase activity in the sample.

The minimum detectable concentration of ALP in serum with an acceptable level of precision was determined as 18 U/l. The manufacturer's determined normal values for adult females aged less than 60 years are 30 – 80 U/l at $30^0$C and 30 – 120 U/l at $37^0$C.

The within-run precision was as follows:

| Serum ALP | Level 1 | Level 2 | Level 3 |
|---|---|---|---|
| Mean (U/l) | 34.1 | 79.6 | 469 |
| SD | 0.45 | 1.64 | 7.55 |
| CV (%) | 1.31 | 2.05 | 1.61 |
| N | 20 | 19 | 20 |

The between-run precision was as follows:

| Serum ALP | Level 1 | Level 2 | Level 3 |
|---|---|---|---|
| Mean (U/l) | 56.2 | 103 | 406 |
| SD | 1.54 | 1.72 | 2.95 |
| CV (%) | 2.74 | 1.67 | 0.73 |
| N | 20 | 20 | 20 |

## 4.7 Laboratory methods (urine)

The following analyses were conducted on acidified urine samples (4.5.2), with the exception of βHCG.

### 4.7.1 Urine pregnancy test

After the urine samples were divided, a commercially available, over-the-counter, pregnancy test for measurement of urinary ßHCG, was carried out in order to exclude pregnancy prior to DXA scanning.

### 4.7.2 Calcium

The same assay was used to calculate urine calcium concentrations as described above for serum calcium.

The intra-assay precision was as follows:



| Urine calcium | Level 1 | Level 2 | Level 3 |
|---|---|---|---|
| Mean (mmol/l) | 1.57 | 2.16 | 3.90 |
| SD | 0.041 | 0.021 | 0.031 |
| CV (%) | 2.28 | 0.91 | 0.79 |
| n | 20 | 20 | 20 |

The inter-assay precision was as follows:

| Urine calcium | Level 1 | Level 2 | Level 3 |
|---|---|---|---|
| Mean (mmol/l) | 1.75 | 2.23 | 3.77 |
| SD | 0.039 | 0.023 | 0.058 |
| CV (%) | 2.23 | 1.04 | 1.54 |
| n | 20 | 20 | 20 |

### 4.7.3 Phosphate

The same assay was used to determine urine phosphate concentration as described for serum phosphate (4.6.3). The manufacturers determined normal range is 0.4 – 1.3 mmol/d 24-hour urine collection. The urine samples for this study were spot urines, i.e. the urines were collected after an overnight fast and were the 2nd void urines of the day. The minimum detectable concentration with an acceptable level of precision was 1.14 mmol/l.

The intra-assay precision was as follows:

| Urine phosphate | Level 1 | Level 2 | Level 3 |
|---|---|---|---|
| Mean (mmol/l) | 9.65 | 32.65 | 63.43 |
| SD | 0.27 | 0.81 | 1.59 |
| CV (%) | 2.78 | 2.49 | 2.52 |
| N | 20 | 20 | 20 |

The inter-assay precision was as follows:

| Urine phosphate | Level 1 | Level 2 | Level 3 |
|---|---|---|---|
| Mean (mmol/l) | 9.15 | 28.8 | 55.1 |
| SD | 0.21 | 0.84 | 1.55 |
| CV (%) | 2.34 | 2.93 | 2.82 |
| N | 20 | 20 | 20 |

### 4.7.4 Creatinine

The same assay was used to determine urine creatinine concentration as described for serum creatinine (4.6.4). The manufacturer's determined normal range was 8.84 – 13.3 mmol/24-hours for 24-hour urine collection. As with urinary phosphate measurements, the urine samples for this study were spot urines, i.e. the urines were collected after an overnight fast and were the 2nd void urines of the day.

The minimum detectable concentration with an acceptable level of precision was determined as 310 µmol/l. Urine was diluted with 1+8 with 0.9% NaCl solution; this was performed automatically using the urinary creatinine programme.

The within-run precision was as follows:

| Urine creatinine | Level 1 | Level 2 | Level 3 |
|---|---|---|---|
| Mean (mmol/l) | 4.46 | 8.93 | 17.75 |
| SD | 0.10 | 0.18 | 0.27 |
| CV (%) | 2.1 | 2.1 | 1.5 |
| n | 88 | 87 | 88 |

The total-run precision was as follows:

| Urine creatinine | Level 1 | Level 2 | Level 3 |
|---|---|---|---|
| Mean (mmol/l) | 4.46 | 8.93 | 17.75 |
| SD | 0.13 | 0.26 | 0.50 |
| CV (%) | 3.0 | 3.0 | 2.8 |
| n | 88 | 87 | 88 |

### 4.7.5 TmP/GFR

The ratio of renal tubular maximum reabsorption rate of phosphate to glomerular filtration rate (TmP/GFR) is a measure of maximum renal tubular phosphate reabsorption in mass per unit volume of glomerular filtrate. It is independent of other factors influencing plasma phosphate concentration, i.e. rate of phosphate efflux from gut, bones, and cells into the extracellular space, and the GFR. As plasma phosphate concentration increases so does the appearance of the ion in urine. When the nephrons have reached their maximum capacity to reabsorb phosphate there is a linear relationship between increase in plasma phosphate and rate of urine excretion.



The Mayo Clinic defines measurement of phosphate reabsorption as the most convenient way to evaluate renal phosphate transport and set the 'theoretical renal phosphate threshold'. This threshold corresponds to the theoretic lower limit of plasma phosphate below which all filtered phosphate would be reabsorbed in the renal tubule. Although direct measurements of parathyroid hormone (PTH), which increases renal phosphate excretion have replaced much of the utility of TmP/GFR measurements, it may still be useful in assessing renal reabsorption of phosphate in a variety of pathological conditions (Mayo Clinic, 2012).

Algorithms derived by Kenny and Glen are used to calculate TmP/GFR in this analysis (Payne, 1998).

Urine and blood phosphate ($U_p$ and $S_p$) concentrations are measured, as are blood and urine creatinine measurements ($U_{cr}$ and $S_{cr}$).

The ratio of phosphate clearance to creatinine clearance gives the fraction of filtered phosphate that is not reabsorbed so appears in the urine (e.g. 0.15 or 15%). When subtracted from 1.0 this gives the fractional tubular reabsorption of phosphate (TRP) (e.g. of 0.85). In general, a low TRP in the presence of hypophosphatemia is indicative of a renal defect in phosphate reabsorption. The equation for TRP is:

$$\mathbf{TRP=1-[(Up/Sp) \times (Scr/Ucr)]}$$

A value less than, or equal to, 0.86 indicates a value lying in the linear part of the excretion curve, suggesting maximum phosphate reabsorption.

Assuming serum (or plasma) phosphate is equal to that found in glomerular filtrate then TRP multiplied by $S_p$ gives the maximum reabsorption per unit volume of filtrate, i.e.:

$$\mathbf{TmP/GFR=TRP \times S_p}$$

If, however, TRP is greater than 0.86 then the value lies on the non-linear part of the derived curve and a different formula is used to calculate TmP/GFR. The assumptions of

Kenny and Glen's algorithms are that $S_p = U_p$, and that $S_{cr}$ is a close proxy of creatinine clearance (Payne, 1998; Bagga *et al*, 2005). The normal tubular reabsorption of phosphate is >80% and the TmP/GFR 2.6 – 4.4 mg/dL (0.80 – 1.35 mmol/l).

### 4.8 Dietary assessment

The full background, design, and results of the dietary assessment component of this study are located in Appendix 6. A summary of baseline key nutrient intake data is presented in Section 5.4. The dietary assessment tool used in this study was the South African MRC Food Frequency Questionnaire (FFQ). This dietary intake tool has been devised to assess dietary intakes of South Africans and is linked to the food composition database called 'Food Finder' dietary analysis programme previously used to estimate habitual dietary intake in metropolitan Johannesburg populations (MRC South Africa, 2002; Zingoni *et al*, 2009). This tool was used at the baseline visit and all participants underwent assessment of dietary intake by FFQ. At the six and 12-month visits, participants were asked a modified dietary questionnaire that enquired into changes in dietary intake, appetite, and dietary habits since the last visit. The aim of this was to identify changes in appetite and dietary, and seasonal, intake patterns and in particular, changes associated with exposure to ART.

### 4.9 Measures of socio-economic status

Given the high rates of unemployment, it was felt that employment status or occupation would not be a good discriminator of socio-economic status (SES). Therefore, at the six month visit the women were asked questions about their educational attainment, (i.e. which school grade they finished) and the household ownership of certain material items such as mobile telephones or microwave ovens; these were then used as a proxy measure of SES. Measures of SES are notoriously difficult to equate to actual living standards in terms of the economic and social components (Sheppard *et al*, 2009). The brief, 10-point measure of household SES used in this study largely mirrors that used in previous South African studies and allowed group comparisons in terms of household assets (ibid.). A further proxy for SES was an assessment of educational attainment to



evaluate if there were group differences. This evaluation of SES was thought to be important as measures of SES are associated with different health outcomes (Griffiths *et al*, 2012) and may influence skeletal health.

### 4.10 Clinically reportable data

Those subjects who had a clinically relevant result, for example a positive HIV-antibody test or a BMD Z-score of <-2.0, were referred to the most appropriate clinical service for ongoing management. Other reportable data included any abnormal biochemical result that was considered clinically relevant. As previously mentioned, underweight and overweight participants were offered dietetic referral (Section 4.3.3), and those with a blood glucose measurement of >11.1 mmol/l (Section 4.5.1.1) were immediately referred to their local clinical service for follow-up.

## 4.11 Data handling and statistical analysis

### 4.11.1 Data handling

All original source documents were examined by me, after completion of each study visit and before the participant left the department, so that any implausible or missing data and discrepancies could be rechecked or collected. On the rare occasion that I was unable to complete this task it was conducted by a trained research assistant (Dimakatso Mafokwane) and then the completed forms rechecked by me within one or two days. This process of 'check out' was an integral part of the study visit and took place with the participant in the room and prior to her being given money for transport. The person completing this process signed and dated the paperwork to ensure that all data had been checked.

A computer database was established at DPHRU by Mat Mainwaring, data manager. All data were singly entered from the primary source material (paper forms) into a Microsoft Access™ 2010 database by experienced data coder research assistants (Jackson Mabasa, Nomses Baloyi, Zandile Lephoto, and Eliza Tsoeu) at DPHRU. Each coder concentrated on a specific questionnaire or data set (e.g. anthropometry) until the whole of that dataset had been entered into the Access database. Further quality checks were carried out by me, which involved cross-checking 20% of all entered data against the primary source material. Any mistakes were passed back to the original data entry research assistant for appropriate correction. Regular meetings were held with the research assistants to explain the rationale of the study and the reason certain data were being collected. All research assistants were encouraged to freely enquire about perceived discrepancies in any document so that these could be addressed immediately as well as to provide an opportunity for ongoing training. Data was emailed to me by Mat Mainwaring and then imported into Microsoft Excel™ 2010 for ease of scrutiny for outliers and obvious errors, then the data were imported into DataDesk.

### 4.11.2 Data transformation

All continuous variables were expressed in their original units (Chapters 5 and 6) and discrete variables were coded as 0 or 1. Continuous variables apart from age were also



transformed into natural logarithms (Ln) to allow for the exploration of proportional relationships between continuous and discrete variables (Chapters 7 and 8). Conversion to Ln is a convenient way of expressing proportional differences between groups. It also allows for a regression analysis in which coefficients provide information about the relative influence of each factor on the dependant variable. A sympercent is derived when the change in dependant variable is expressed in Ln and then multiplied by 100, a difference between groups or timepoints corresponds closely to the percentage difference between them, i.e. (difference/mean) x 100 (Prentice *et al*, 1994; Cole, 2000; Jarjou *et al*, 2010). If the independent variable is continuous the coefficient x 100 corresponds to the percentage increment per 100% change in the variable. The transformation of data into Ln also normalises skewness.

Baseline data are presented in absolute terms for ease of interpretation of actual values. For all longitudinal analysis data are presented in absolute terms (e.g. mmol/l) and as % difference from baseline.

### 4.11.3 Statistical analysis

All data were analysed using DataDesk 6.1.1 (Data Description Inc, Ithaca, NY). Summary statistics were presented as mean (±standard deviation (SD)) or median (interquartile range (IQR)), depending on the distribution. Categorical data were compared using $Chi^2$ where appropriate using STATA 11.1 (STATACorp LP, Texas). When there was low cell frequency Freeman-Halton extension to 3*2 tables of Fisher exact probability test was used instead of $Chi^2$. Comparisons were made between the three groups of women using hierarchical linear models; ANOVA (or ANCOVA) and Scheffé *post hoc* tests were used to compare group means (standard error (SE)). Bone mineral data were fully adjusted for age, weight, height, and bone area where appropriate (see Sections 4.11.4, 2.2.7.5 ). A p value of ≤0.05 was considered to be statistically significant.

To evaluate individual SA-BMC values, a residual value for each subject was obtained from the relevant model and added to the group mean value.

Preliminary plots of the relationship between fat mass and lean mass in this sample population demonstrated non-linearity. Regression of fat mass on lean mass in the HIV-negative control group with data in natural logarithms gave a power exponent of 2.05 ± 0.14 (SE), indicating that fat mass-to-lean mass$^2$ best described the relationship in this population. The exponent was similar when the entire data set were included in the model; 1.98± 0.14. The exponent did not change significantly at the 6- and 12-month time points; 2.25± 0.14, 2.08± 0.16 respectively. Consequently, a fat mass-to-lean mass$^2$ term was used to describe differences in body composition between the groups, and logarithmic regression was used to adjust fat mass for lean mass in statistical models.

SD-scores (SDS) for aBMD were generated using Nref as the reference population (ref) against which the SDS for each individual HIV-positive woman (i) was derived as follows: [(aBMDi– mean aBMDref )/SDref.]

Some women acquired HIV infection during the course of the study. Some in Ppres group did commence ART, and some in Plow group did not commence ART as planned (see Section 6.1.2). However, as this was an observational study and not a trial, a decision was made that subjects should be analysed based on the original group that they were assigned to. However, as the major hypothesis (see Section 1.6) was based on ART exposure a decision was made to also analyse the data based on exposure to ART in a binary No/Yes fashion. To consider a possible association with ART exposure a *post hoc* division of HIV-positive women into those ART-exposed and ART-unexposed was performed. These formed two approximately equal groups. This division allowed consideration of the question 'did ART exposure influence outcomes such as change in bone mineral, body composition or vitamin D status?' The nature of the division was dichotomous (Yes or No). Including duration of exposure did not provide further discrimination, largely because most that were exposed to ART initiated had ART close to baseline, and this variable was excluded in the models presented in Chapter 8



### 4.11.4 Model design

The effect of HIV status and exposure to ART treatment on bone mineral status at each site measured, (i.e. total hip, femoral neck, lumbar spine, and whole body) was examined in the following ways:

a. Effect on BMC over time;
b. Effect on bone area (BA) over time;
c. Effect on aBMD (aBMD=BMC/BA) over time;
d. Effect on BMC (and so aBMD) after full correction for age, height, weight, and BA, i.e. size adjusted BMC (SA-BMC), to allow for effects on the skeleton independent of bone and body size (Prentice *et al*, 1994);
e. Where appropriate, effect on BA after correction for age, height, and weight.

Weight and height were included in the models separately rather than using derived measures such as BMI as these, separately, better discriminate effects of body composition on bone than when used as a composite measure (Cole *et al*, 1992). This is because both BMC and aBMD are positively and independently associated with height and weight (ibid.). The effect of HIV-status and exposure to ART treatment on body composition and vitamin D status at each time point was examined.

Comparisons of changes in BMC, BA, aBMD, and SA-BMC, body composition and 25(OH)D between the 3 groups were examined using simultaneous, multiple, linear regression. For consistency, all variables of interest were included in an initial full model for the regression analysis, followed by backward elimination for non-significant factors ($p>0.05$) (see Figure 4-11). The least significant factors were removed first to produce a final parsimonious model. Interaction terms (see Section 4.11.4.6) remained in the model regardless of their significance. Where appropriate, baseline values of the independent variable were included in the regression model to adjust for regression toward the mean.

#### 4.11.4.1 Regression analyses

Regression analysis is a statistical technique for estimating the relationships among variables. It includes many techniques for modelling and analysing several variables, when the focus is on the relationship between a dependent variable and one or more independent variables. Regression analysis demonstrates how the typical value of the dependent variable changes when any one of the independent variables is varied, while the other independent variables are held fixed.

In order to examine observed changes in variables a series of regressions, and linear regressions and group-by-variable interactions were scrutinised. This was intended to allow exploration of predictors of key variables such as the ability of fat mass to predict vitamin D status (see Section 5.7.1).

The linear models illustrated in Figure 4-11 to Figure 4-14 combined features of regression, ANOVA and ANCOVA. ANCOVA is a mix of ANOVA and regression analysis.



**Figure 4-11 An example of a full linear model in DataDesk**

**DESIGN**

**Dependent variables**

| Name | Code |
|---|---|
| ABMCNeck | ABk |

Type of analysis: OLS ANOVA

**Factors**

| Name | Code | Nested in | F/R | Kind |
|---|---|---|---|---|
| Agroup | Agp | ( ) | Fix | Disc |
| Aage | Aag | ( ) | Fix | Cont |
| Aheight | Aht | ( ) | Fix | Cont |
| Aweight | Awt | ( ) | Fix | Cont |
| AAreaNeck | ANk | ( ) | Fix | Cont |

Partial (Type 3) Sums of Squares

Interactions up to 1 - way

**No Modifications**

**RESULTS**

**General Results**

247 total cases of which 5 are missing

ANOVA

Analysis of Variance For **ABMCNeck**
No Selector
247 total cases of which 5 are missing

| Source | df | Sums of Squares | Mean Square | F-ratio | Prob |
|---|---|---|---|---|---|
| Const | 1 | 4633.76 | 4633.76 | 18251 | ≤ 0.0001 |
| Agp | 2 | 1.23086 | 0.61543 | 2.424 | 0.0908 |
| Aag | 1 | 0.537258 | 0.537258 | 2.1161 | 0.1471 |
| Aht | 1 | 0.123123 | 0.123123 | 0.48494 | 0.4869 |
| Awt | 1 | 17.6075 | 17.6075 | 69.35 | ≤ 0.0001 |
| ANk | 1 | 7.34337 | 7.34337 | 28.923 | ≤ 0.0001 |
| Error | 235 | 59.6652 | 0.253895 | | |
| Total | 241 | 93.4349 | | | |

**Results for factor** Agp

(DataDesk model to examine SA-BMC of the femoral neck. This linear model (i.e. ANCOVA) is a combination of ANOVA and regression analysis)

#### 4.11.4.2 Analyses of variance (ANOVA)

Analysis of variance (ANOVA) was used to ascertain whether two or more groups had the same mean value (i.e. as tests for uniformity of mean values). ANOVA is a parametric method based on an assumption of normality, i.e. that data are from normally distributed populations with uniform variance even in the face of different mean values. In reality the ANOVA works well even when the normality assumption is imperfectly satisfied (Kirkwood *et al*, 2005).

This comparison statistical testing follows an F distribution. The F-statistic tests the null hypothesis of the means of the groups being equal against an alternative hypothesis of at least one group mean not being equal. Larger F-values suggest that the mean values vary more than anticipated for measures between different groups. Conversely, smaller F-values support the null hypothesis that the mean group values are not discernibly different between groups (Stear, 1998). The *p* value for the ANOVA shows the probability of observing an F-value as large as the one observed if the null hypothesis is true. A small *p* value suggests that the F-value observed did not occur by chance. In this study ANOVA was used to test for differences between the different study groups, i.e. Nref, Ppres, and Plow.

#### 4.11.4.3 Scheffé *post hoc* testing

These *post hoc* tests examine differences between the predicted values of the dependant variables in pairs of groups by comparing group means. Multiple testing involves a high probability of generating type 1 errors, i.e. those that appear to meet the criterion of significance by chance. Scheffé *post hoc* testing has the advantage of controlling for and minimising the occurrence of type 1 errors. In this study the type 1 error rate was set at 5%. When comparing group means of 3 or more groups that are not ordered there is no 'best approach' for comparing group means (Altman, 1999). One possibility is to compare each pair of means in turn; the problem associated with this approach is that multiple significance testing gives a high probability of finding a significant result by chance. Each test has a 5% chance of a false positive result. Scheffé is aimed at controlling this overall type 1 error rate and was chosen because of its conservative nature (Altman, 1999). It is preferable in comparison with other tests such as the Tukey–Kramer method, which examines pairwise differences, as it tends to give narrower confidence limits.



**Figure 4-12 An example of Scheffé *post hoc***

**DESIGN**

**Dependent variables**

| Name | Code |
|---|---|
| ABMCNeck | ABk |

Type of analysis: OLS ANOVA

**Factors**

| Name | Code | Nested in | F/R | Kind |
|---|---|---|---|---|
| Agroup | Agp | () | Fix | Disc |
| Aage | Aag | () | Fix | Cont |
| Aheight | Aht | () | Fix | Cont |
| Aweight | Awt | () | Fix | Cont |
| AAreaNeck | ANk | () | Fix | Cont |

Partial (Type 3) Sums of Squares

Interactions up to 1 - way

**No Modifications**

**RESULTS**

**General Results**

247 total cases of which 5 are missing

ANOVA

Analysis of Variance For **ABMCNeck**
No Selector
247 total cases of which 5 are missing

| Source | df | Sums of Squares | Mean Square | F-ratio | Prob |
|---|---|---|---|---|---|
| Const | 1 | 4633.76 | 4633.76 | 18251 | ≤ 0.0001 |
| Agp | 2 | 1.23086 | 0.61543 | 2.424 | 0.0908 |
| Aag | 1 | 0.537258 | 0.537258 | 2.1161 | 0.1471 |
| Aht | 1 | 0.123123 | 0.123123 | 0.48494 | 0.4869 |
| Awt | 1 | 17.6075 | 17.6075 | 69.35 | ≤ 0.0001 |
| ANk | 1 | 7.34337 | 7.34337 | 28.923 | ≤ 0.0001 |
| Error | 235 | 59.6652 | 0.253895 | | |
| Total | 241 | 93.4349 | | | |

**Results for factor** Agp

Coefficients

Expected Cell Means

Scheffe Post Hoc Tests

| | Difference | std. err. | Prob |
|---|---|---|---|
| 2 - 1 | -0.0538466 | 0.08086 | 0.801322 |
| 3 - 1 | 0.131994 | 0.08451 | 0.298019 |
| 3 - 2 | 0.18584 | 0.08617 | 0.0999831 |

(Full model in DataDesk (to examine SA-BMC of the femoral neck) demonstrating Scheffé *post hoc* tests)

#### 4.11.4.4 Analysis of covariance (ANCOVA)

Continuous variables are described as covariates. ANCOVA allows for analyses of both discrete and continuous factors, whereas ANOVA is limited to discreet factors. ANCOVA is similar to ANOVA but allows the independent, predictor, variable to be a combination of discrete and continuous. Where binary variable are used (0/1) ANCOVA is similar to

regression analysis. ANCOVA enables the examination of linear trends in dependant variables across the range of the covariate (continuous factor). For instance, I was able to investigate the influences of continuous variables, e.g. weight or BMI, and discrete groups (e.g. subject group) on change in aBMD or vitamin D status. DataDesk sets all factors to be discrete by default, therefore the covariates have to be amended to 'continuous' prior to analysis.

#### 4.11.4.5 Nesting

The experimental design of this study included nested factors; these are factors whose levels are different for each level of some other discrete factor. Nested factors are ordinarily random and this allows separation of the, in this case, individual effect from the group effect. Nesting refers to the design wherein each main effect can be nested in the previous one so that the nesting forms a hierarchy. DataDesk has a function that allows nesting of repeated measures so that one factor is linked to another factor. In this analysis, individual subject identification (ID) can be nested within the group (Nref, Ppres, or Plow). This ensures that linked factors (e.g. individual subject ID and group) are controlled for in the analysis.

**Figure 4-13 An example of nesting**

**DESIGN**

**Dependent variables**

| Name | Code |
|---|---|
| hL25Dnmol/L | h2L |

Type of analysis: OLS ANOVA

**Factors**

| Name | Code | Nested in | F/R | Kind |
|---|---|---|---|---|
| TpT0_6_12 | T02 | () | Fix | Disc |
| IDh | IDh | (GRh) | Ran | Disc |
| GRPh | GRh | () | Fix | Disc |

Partial (Type 3) Sums of Squares

Interactions up to 1 - way

**No Modifications**

**RESULTS**

**General Results**

741 total cases of which 69 are missing

ANOVA

Analysis of Variance For **hL25Dnmol/L**
No Selector
741 total cases of which 69 are missing

| Source | df | Sums of Squares | Mean Square | F-ratio | Prob |
|---|---|---|---|---|---|
| Const | 1 | 10814.2 | 10814.2 | 45721 | ≤ 0.0001 |
| T02 | 2 | 5.40137 | 2.70069 | 43.471 | ≤ 0.0001 |
| IDh | 244 | 57.712 | 0.236525 | 3.8072 | ≤ 0.0001 |
| GRh | 2 | 0.0604749 | 0.0302374 | 0.12784 | 0.8801 |
| Error | 423 | 26.2791 | 0.0621256 | | |
| Total | 671 | 89.3295 | | | |

**Results for factor** T02

(ID (IDh) nested within Group (GRPh))



#### 4.11.4.6 Interactions

An interaction refers to the combined effect of two or more factors unaccounted for by individual factors. An interaction assesses whether the response, as measured by one of the variables, changes at different levels of the other factor.

In DataDesk's linear model function a command allows for interaction terms to be created in ANOVA or ANCOVA models. In this study, the relationship between the groups (Nref, Ppres, or Plow or ART-unexposed/exposed) and the different time points was the main interaction assessed (0, 6, and 12 months) in order to determine whether this interaction was more important than each factor individually. This made it possible to determine whether the group effect influenced the time effect on the variables of interest or vice versa, that is if the time effect influenced group effect.

**Figure 4-14 An example of an interaction term**

**DESIGN**

**Dependent variables**

| Name | Code |
|---|---|
| hL25Dnmol/L | h2L |

Type of analysis: OLS ANOVA

**Factors**

| Name | Code | Nested in | F/R | Kind |
|---|---|---|---|---|
| TpT0_6_12 | T02 | () | Fix | Disc |
| IDh | IDh | (GRh) | Ran | Disc |
| GRPh | GRh | () | Fix | Disc |

Partial (Type 3) Sums of Squares

Custom interactions

| Source | F/R | max df | EMS | F-Denom |
|---|---|---|---|---|
| Const | - | 1 | IDh+Const | IDh |
| T02 | F | 2 | T02 | Error |
| IDh | R | 738 | IDh | Error |
| GRh | F | 2 | GRh+IDh | IDh |
| T02*GRh | F | 4 | T02*GRh | Error |
| Error | R | (6) | | |
| Total | | 740 | | |

**No Modifications**

**RESULTS**

**General Results**

741 total cases of which 69 are missing

ANOVA

Analysis of Variance For **hL25Dnmol/L**
No Selector
741 total cases of which 69 are missing

| Source | df | Sums of Squares | Mean Square | F-ratio | Prob |
|---|---|---|---|---|---|
| Const | 1 | 10814.2 | 10814.2 | 45706 | ≤ 0.0001 |
| T02 | 2 | 5.1915 | 2.59575 | 42.595 | ≤ 0.0001 |
| IDh | 244 | 57.7317 | 0.236605 | 3.8825 | ≤ 0.0001 |
| GRh | 2 | 0.0489007 | 0.0244504 | 0.10334 | 0.9019 |
| T02*GRh | 4 | 0.744959 | 0.18624 | 3.0561 | 0.0168 |
| Error | 419 | 25.5342 | 0.0609407 | | |
| Total | 671 | 89.3295 | | | |

**Results for factor** T02

(Timepoint (T02)*Group (GRh) interaction term)

#### 4.11.4.7 Per protocol and *post hoc* analysis

The analysis of the effect of HIV infection on bone mineral status at each site measured as well as on vitamin D status was conducted on a Per Protocol Analysis, so that each individual was analysed in the original group to which she was assigned. Furthermore, as described above (Section 4.11.3) a *post hoc* analysis was conducted to examine the potential effect of ART exposure on bone, body composition, and vitamin D measures.



This meant that all subjects were compared according to their original group allocation as well as by their ART exposure status in the HIV-positive women.

Since this study was designed to detect differences between groups in change over time, the main reason for this approach was to preserve the original study design that was powered to detect differences in changes between groups. Even in these exceptional cases subjects were kept in the group that they were originally assigned to for analysis to maintain the integrity of the study. Since this was not a clinical trial, it was appropriate to track changes in individuals in all the complexities of their circumstances. To overcome any bias introduced by this approach, data were analysed in several different ways to see if reordering the grouping affected the significance of the results. The data were reanalysed based on, for example, $CD_4$ counts less than or greater than 200 $x10^6$/l or 350 $x10^6$/l and based on exposure to ART or not. Reanalysing the data in these ways did not alter the interpretation of the results, so a decision was made to present the data as a Per Protocol Analysis with a further *post hoc* analysis of the HIV-positive women. The effect of ART exposure was analysed as a binary variable (see Section 4.11.3). Using ART exposure as a continuous variable to represent cumulative dose of ART did not affect the interpretation of results.

# 5 Baseline results

The results of this cross-sectional analysis are in press in *Osteoporosis International* (MM Hamill, KA Ward, JM Pettifor, SA Norris, and A Prentice (Appendix 7)) and *Public Health Nutrition* (SV Wrottesley, LL Micklesfield, MM Hamill, GR Goldberg, A Prentice, JM Pettifor, SA Norris, and AB Feeley (Appendix 6)).

## 5.1 Overview

Weight, BMI, waist circumference, fat mass, and fat:lean[2] ratio were significantly greater in Nref and Ppres than Plow women. Hip circumference was significantly greater in Nref and Ppres than Plow. Nref had a significantly smaller waist:hip ratios than Ppres and Plow. The percentage overweight was 65% (in Nref and Ppres) compared to 44% in Plow (Table 5-6 and Table 5-8).

There were no significant differences in measures of bone mineral status before or after fully correcting for weight and height between the three groups (Table 5-10). Likewise, there were no significant between-group differences in vitamin D status (Table 5-12). Plow women had significantly lower serum albumin, higher serum phosphate, and greater TMP/GFR than Ppres and Nref (Table 5-13). Nref and Ppres were not different in any measure of body composition, bone mineral, or vitamin D status, but Ppres had lower serum albumin and higher serum phosphate and TMP/GFR than Nref.

## 5.2 Recruitment

The initial power calculations were based on a total of 241 but the number recruited was greater to allow for potential loss to follow-up (see Section 3.5). In order to account for a dropout rate of greater than 10%, whilst still retaining the necessary power, the study recruited n=247; 98 controls (Nref) and 149 HIV-positive subjects. The HIV-positive group comprised 74 Ppres and 75 Plow. Ppres were women with a $CD_4$ count ≥350 x$10^6$ cells/l, who underwent regular clinical review and were not anticipated to require ART during the course of the study. Plow were women with low $CD_4$ counts (≤200 x$10^6$ cells/l) who were due to commence ART as soon as feasible via their regular clinical provider.



It was hoped that the three groups would be recruited in parallel, however, in practice the Nref women group finished recruitment first, followed by the Ppres and the Plow.

## 5.3 Subject characteristics

Table 5-1 describes the subject characteristics of the women at baseline. As expected from the study design, mean ±SD $CD_4$ count (x $10^6$ cells/l) in the Ppres group was significantly higher than that in the Plow group (412 ±91 and 161 ±69 respectively, p<0.0001).

Mean age ±SD was 32.1 ±7.2 years with HIV-positive women being significantly older on average than HIV-negative women (p <0.01), although the age ranges were similar in the three groups (18-49, 22-48 and 19-47 years in Nref, Ppres and Plow respectively). Median (IQR) gravidity was 2 (1;3) with both Ppres and Plow having a higher median gravidity compared to Nref (p≤0.0001). Current hormonal contraception usage was similar across the groups with about a third of women reporting current usage.

**Table 5-1 Subject characteristics**

| Group | Nref *n*=98 | Ppres *n*=74 | Plow *n*=75 | Group effect ANOVA *P* = |
|---|---|---|---|---|
| Age (years) | 30.0 (8.1) | 33.5 (6.1)[A] | 33.4(6.5)[B] | 0.001 |
| $CD_4$ count x $10^6$ cells/l | ND | 412 (91)[3] | 161 (69)[c] | <0.0001 |
| Min | NA | 240 | 18 | |
| Max | NA | 604 | 275 | |
| Gravidity median (IQR) | 1 (0;2) | 2 (2;3) | 2 (1;3) | NS |
| Range | 0-5[1] | 0-6[A] | 0-6[B,3] | |
| Current hormonal contraceptive use (%) | 34.0 (35.4)[2] | 26.0 (36.6)[4] | 25.0 (33.3) | 0.92 |

All values are Mean ±SD unless stated. IQR, interquartile range; NA, not applicable; ND, not determined; NS, non-significant
Letters are used to indicate significance of between-group differences as tested by ANOVA/Scheffé
[A] Significantly different from Nref P ≤0.01.
[B] Significantly different from Nref P ≤0.01.
[C] Significantly different from Ppres P ≤0.001.
[1]n=97, [2]n=96, [3]n=74, [4]n=71

### 5.3.1 Socio-economic status/lifestyle factors

Individual socio-economic status was evaluated at the 6-month study visit but is presented here as baseline characteristics for context. Data are presented for 220/247 participants (89%) (Table 5-2). The number in each group reflects the number returning for the 6-month visit, i.e. 84%, 99%, and 87% for groups Nref, Ppres, and Plow respectively (see Sections 6.1 and 6.2).

**Table 5-2 Measures of household assets**

| Group | Nref *n*=82 | Ppres *n*=73 | Plow *n*=65 | *P*= |
|---|---|---|---|---|
| **Cellular telephone** | | | | |
| No/Yes (%N;Y) | 1/81 (9; 81) | 0/73 (0; 100) | 1/64 (1; 99) | 0.8* |
| **Microwave** | | | | |
| No/Yes (%N;Y) | 13/69 (16; 84) | 13/60 (18; 82) | 19/46 (23; 71) | 0.1 |
| **Video machine** | | | | |
| No/Yes (%N;Y) | 13/69 (16; 84) | 16/57 (22; 78) | 12/53 (19; 82) | 0.6 |
| **Telephone** | | | | |
| No/Yes (%N;Y) | 66/16 (80; 20) | 63/10 (86; 14) | 59/6 (91; 9) | 0.2 |
| **Washing machine** | | | | |
| No/Yes (%N;Y) | 34/48 (41; 59) | 31/42 (43; 57) | 37/28 (57; 43) | 0.1 |
| **Fridge** | | | | |
| No/Yes (%N;Y) | 1/80 (1; 99) | 5/68 (7; 93) | 13/52 (20; 80) | 0.0003* |
| **Car** | | | | |
| No/Yes (%N;Y) | 52/30 (63; 37) | 53/19 (74; 26) | 42/23 (65; 35) | 0.4 |
| **Radio** | | | | |
| No/Yes (%N;Y) | 3/79 (4; 96) | 4/69 (6; 94) | 5/60 (8; 92) | 0.6* |
| **TV** | | | | |
| No/Yes (%N;Y) | 3/79 (4; 96) | 2/71 (3; 97) | 1/64 (2; 98) | 0.9* |
| **Electricity** | | | | |
| No/Yes (%N;Y) | 2/80 (2; 98) | 2/71 (3; 97) | 4/60[a] (6; 94) | 0.5 |

N are different from baseline because data obtained at 6 months.
[a]Less than number in the group as item missed on questionnaire.
* indicates use of Freeman-Halton extension to 3*2 tables of Fisher exact probability test instead of $chi^2$ test due to low cell frequency.

**Table 5-3 Individual educational attainment**

| **Educational achievement** | **Level/school grade completed** | Nref *n*=82 | % | Ppres *n*=73 | % | Plow *n*=65 | % |
|---|---|---|---|---|---|---|---|
| 2 | Nil | 0 | 0.0 | 0 | 0.0 | 1 | 1.5 |
| 3 | 1 – 2 | 1 | 1.2 | 2 | 2.7 | 0 | 0.0 |
| 4 | 3 – 5 | 1 | 1.2 | 1 | 1.4 | 3 | 4.6 |
| 5 | 6 – 7 | 1 | 1.2 | 5 | 6.8 | 4 | 6.2 |
| 6 | 10 | 7 | 8.5 | 8 | 11.0 | 12 | 18.5 |
| 7 | 11 | 14 | 17.1 | 27 | 37.0 | 21 | 32.3 |
| 8 | 12 (matriculation) | 53 | 64.6 | 28 | 38.3 | 22 | 33.8 |
| 9 | Post matriculation | 4 | 4.9 | 1 | 1.4 | 1 | 1.5 |
| 10 | Diploma | 1 | 1.2 | 1 | 1.4 | 1 | 1.5 |



There were no significant differences between groups in measures of household possessions except for possession of a fridge, which was significantly lower in Plow. There were group differences in educational attainment $Chi^2$=23.1, p=0.001. This was significantly higher in Nref compared with Ppres and Plow, because the control group had many more subjects who had attained a matriculation qualification. Educational attainment for the $Chi^2$ test was collapsed into 4 groups (2 – 5, 6, 7, 8 – 10).

There were no significant differences in self-reported current smoking, current alcohol use, or history of ever-fracture (Table 5-4).

**Table 5-4 Smoking, alcohol intake, and fracture history**

| | | Nref *n*=98 | Ppres *n*=74 | Plow *n*=75 |
|---|---|---|---|---|
| Current smoker | Y | 10 | 13 | 8 |
| | N | 88 | 87 | 92 |
| | ND | 2 | 0 | 0 |
| Current alcohol use | Y | 20 | 26 | 16 |
| | N | 80 | 74 | 84 |
| | ND | 0 | 0 | 0 |
| History of fracture | Y | 24 | 26 | 13 |
| | N | 72 | 73 | 85 |
| | ND | 4 | 1 | 2 |

All values are percentages.
n= number; N, No; ND, Not documented; Y, Yes.
There were no significant group differences by $Chi^2$ testing.

## 5.4 Dietary data

At baseline there were no significant group differences in the intakes of macronutrients and micronutrients relevant to bone health (Table 5-5). United States Dietary Reference Intake (DRIs) for energy and macronutrients were selected for assessing the intakes of the groups compared to recommendations as these are most commonly used in South Africa and therefore are the most useful for comparison with other published data (Trumbo *et al*, 2002). The data from the women in the study were therefore compared with the US Estimated Energy Requirement (EER) and the Recommended Dietary Allowance (RDA) for protein and carbohydrate in adult women aged 19 to 50 years. The median intakes for carbohydrate, protein, fat, and fibre as proportions of total energy

intake were calculated and compared with the Acceptable Macronutrient Distribution Ranges (AMDR).

Energy, carbohydrate, and protein intakes were high across the groups when compared to DRIs of 10.1 MJ (EER), 130g (RDA) and 46g (RDA) respectively (Trumbo *et al*, 2002). None of the components of dietary intake were different between the three groups. Dietary intake exceeded the EER, the RDA for carbohydrate and protein across all three study groups. Carbohydrate, protein, fat, and fibre contributed to approximately 54%, 11%, 30%, and 4%, respectively of total energy intake across all three groups. Intakes, as percentages of total energy intake, were therefore within the AMDR for carbohydrate (45 – 65%), protein (10 – 35%), and fat (20 – 35%) for all groups. Approximately 8%, 5%, and 21% of subjects in the sample had carbohydrate, fat, and protein intakes, respectively, below the acceptable range, while 5% and 19% had carbohydrate and fat intakes, respectively, above the acceptable range. Alcohol contributed to less than 1% of total energy intake in all groups (Wrottesley *et al*, 2013).

Median intakes of calcium and vitamin D were below the recommended intakes of 1000mg (RDA) and 15mcg (RDA) respectively (Institute of Medicine, 2010). Intakes of phosphorus, magnesium, and zinc exceeded the recommended intakes of 700 mg, 310 – 320 mg, and 8 mg (RDAs) respectively (Institute of Medicine, 2001).



**Table 5-5 Daily nutrient intakes**

| Nutrient intake /per day | Nref Median (IQR) *n*=98 | Ppres Median (IQR) *n*=74 | Plow Median (IQR) *n*=75 | Group effect ANOVA P = |
|---|---|---|---|---|
| Energy (MJ) | 11.0 (8.8; 14.1) | 12.7 (10.3; 16.1) | 11.5 (8.9; 14.7) | 0.5 |
| Total digestible carbohydrate (g) | 392 (329; 459) | 404 (339; 503) | 388 (331; 487) | 0.2 |
| Total fat (g) | 86 (68; 123) | 101 (71; 132) | 86 (59; 128) | 0.8 |
| Total protein (g) | 74 (61; 95) | 87 (62; 113) | 74 (52; 106) | 0.4 |
| Alcohol (g) mean (SD) | 2.8 (9.8) | 3.8 (10.1) | 1.9 (7.9) | 0.5 |
| Calcium (mg) | 652 (428; 827) | 632 (474; 899) | 532 (386; 787) | 0.9 |
| Phosphorus (mg) | 1189 (968; 1493) | 1323 (984; 1870) | 1168 (845; 1711) | 0.5 |
| Magnesium (mg) | 332 (257; 401) | 377 (288; 506) | 340 (249; 466) | 0.2 |
| Zinc (mg) | 10 (7; 13) | 12 (8; 16) | 10 (7; 15) | 0.3 |
| Vitamin D (mcg) | 4 (3; 6) | 5 (3; 7) | 4 (2; 6) | 0.2 |

Values are median (IQR) unless stated. There were no significant group differences.

## 5.5 Anthropometry

There were no significant differences in height between the three groups, although Nref tended to be shorter. Nref and Ppres were significantly heavier than Plow but not different from each other. Median (IQR) BMI (kg/m$^2$) was 27.3 (23.1;31.7), 27.8 (23.3;32.3), and 23.5 (20.5;27.0) in Nref, Ppres, and Plow respectively with Plow being significantly lower than the other groups. The percentage overweight and obese (BMI> 25 kg/m$^2$) in Nref, Ppres, and Plow were 65%, 65%, and 44%. The percentage underweight (BMI<18.5 kg/m$^2$) was 4%, 1%, and 11% respectively; Plow had a significantly lower prevalence of overweight and/or obesity, and higher prevalence of underweight than the other groups (Table 5-6).

Plow had significantly smaller waist circumference than Ppres, and smaller hip circumference than Nref and Ppres. However, the mean waist:hip ratio (WHR) was lowest in the Nref group compared with Ppres and Plow suggesting that both Ppres and Plow had different fat distribution compared to Nref. It is important to recognise this difference in WHR was in ART-naïve women, as frank lipodystrophy and subtle changes in body composition are most often attributed to ART. Table 5-7 demonstrates the size effect differences in anthropometric measures between the three groups.

**Table 5-6 Anthropometry**

| | Nref *n*=98 | Ppres *n*=74 | Plow *n*=75 | Group effect ANOVA *P* = |
|---|---|---|---|---|
| Height (cm) | 157.6 (5.9) | 159.4 (5.9) | 159.2 (5.3) | 0.06 |
| Weight (kg) | 69.7 (17.0) | 72.0 (17.4) | 62.3 (15.2)$^{A,B}$ | 0.0009 |
| BMI (kg/m$^2$) median (IQR) | 27.3 (23.1;31.7) | 27.8 (23.3;32.3) | 23.5 (20.5;27.0)$^{A,B}$ | 0.0002 |
| ≥25 (%) | 65 | 65 | 44 | |
| >24.9, <30 (%) | 35 | 28 | 28 | |
| >30 (%) | 30 | 37 | 16 | |
| <18.5 (%) | 4 | 1 | 11 | |
| Waist circumference (cm) | 86.5 (14.2) | 90.2 (15.2)[1] | 83.1 (13.9)$^{B,2}$ | 0.01 |
| Hip circumference (cm) | 107.4 (13.8) | 107.5 (13.9)[1] | 98.4 (13.4)$^{A,B,2}$ | <0.0001 |
| Waist: Hip ratio (cm/cm) | 0.80 (0.07) | 0.84 (0.08)$^{A,1}$ | 0.84 (0.06)$^{A,2}$ | 0.0003 |

All values are Mean (SD) unless indicated.
cm, centimetres; IQR, interquartile range; kg, kilograms.
Letters are used to indicate significance of between-group differences as tested by ANOVA/Scheffé.
[A] Significantly different from Nref *P* ≤0.01.
[B] Significantly different from Ppres *P* ≤0.01.
[1] n=73
[2] n=74



**Table 5-7 Percentage differences in anthropometric measures**

| Variable | Group | % (SE) difference |
|---|---|---|
| Weight (Kg ) | Ppres-Nref | 3.4 (3.6) |
| | Plow-Nref | -11.2 (3.5) |
| | Plow-Ppres | -11.6 (3.8) |
| Median BMI (kg/m$^2$) (IQR) | Ppres-Nref | 1.1 (3.4) |
| | Plow-Nref | -13.4 (3.4) |
| | Plow-Ppres | -14.5 (3.6) |
| Waist circumference (cm) | Ppres-Nref | 4.1 (2.5) |
| | Plow-Nref | -4.0 (2.4) |
| | Plow-Ppres | -8.1 (2.6) |
| Hip circumference (cm) | Ppres-Nref | 0.2 (2.0) |
| | Plow-Nref | -8.8 (1.9) |
| | Plow-Ppres | -9.0 (2.1) |
| Waist: Hip ratio (cm/cm) | Ppres-Nref | 4.0 (1.3) |
| | Plow-Nref | 4.8 (1.3) |
| | Plow-Ppres | 0.8 (1.4) |

All values are %(SE) differences.
cm, centimetres; IQR, interquartile range; kg, kilograms; m, metres.

### 5.5.1 Body composition

There were significant differences in fat mass with Nref and Ppres women having significantly higher fat mass than Plow women (p≤0.001). Although lean mass was also higher in Ppres than Plow women (p=0.005), Nref and Ppres had a higher fat mass to lean mass$^2$ ratio than the Plow group (p=0.003) (Table 5-8). When fully adjusting for lean mass using log-log regression, the Plow group had significantly lower fat mass for their lean mass than the other two groups; for each unit of lean mass the Plow group had a mean difference ±SE of 16 ±5% less fat than the Ppres group, (p=0.02), and 21 ±5% less fat than the Nref group, (p=0.0002) (see Section 4.11.3). The size effect differences in fat mass, lean mass, and fat:lean$^2$ between the three groups is demonstrated in Table 5-9. There were no significant differences in Ppres from Nref in any measure of body composition.

**Table 5-8 DXA derived measures of body composition**

| | Nref *n*=98 | Ppres *n*=74 | Plow *n*=75 | Group effect ANOVA *P* = |
|---|---|---|---|---|
| WBLH fat (kg) | 26.1 (11.5) | 26.1 (9.8)[1] | 19.7 (9.3) [A,B,2] | <0.0001 |
| WBLH lean (kg) | 38.3 (60.8) | 39.5 (62.4)[1] | 36.4 (48.1)[B,2] | 0.005 |
| Fat:lean$^2$ (kg/kg$^2$) | 17.3 (4.8) | 15.9 (4.6)[1] | 14.6 (5.5)[A,C,2] | 0.02 |

All values are Mean (SD) unless indicated. WBLH, whole body less head.
Letters are used to indicate significance of between-group differences as tested by ANOVA/Scheffé.
[A] Significantly different from Nref $P \leq 0.001$.
[B] Significantly different from Ppres $P \leq 0.01$.
[C] Significantly different from Ppres $P \leq 0.05$.
[1] n=73
[2] n=74

**Table 5-9 Percentage differences in fat mass, lean mass, and fat:lean$^2$**

| Variable | Group | % (SE) difference |
|---|---|---|
| Fat (kg) | Ppres-Nref | 1.2 (6.8) |
| | Plow-Nref | -30.3 (6.8) |
| | Plow-Ppres | -31.4 (7.2) |
| Lean (kg) | Ppres-Nref | 3.3 (2.3) |
| | Plow-Nref | 4.7 (2.3) |
| | Plow-Ppres | -7.9 (2.5) |
| Fat:lean$^2$ | Ppres-Nref | -5.3 (5.0) |
| | Plow-Nref | -20.9 (5.0) |
| | Plow-Ppres | -15.6 (5.4) |

All values are %(SE) differences.

## 5.6 Bone mineral status

The bone values at all sites measured at baseline are presented in Table 5-10. As described in Section 4.11.4, these were bone mineral content (BMC, g), bone area (BA, cm$^2$), bone mineral density (BMD g/cm$^2$), and size-adjusted BMC (SA-BMC, g).

The influence of HIV status, age, and anthropometric variables were investigated using linear models. Models were built up to examine the effects of potential influences on BMC, BA, BMD, and SA-BMC for each variable. Additionally, the effects of current cigarette smoking, current alcohol use, and previous history of fracture were built into the model. Therefore results are expressed as:

1. Unadjusted comparisons between three groups (Nref, Ppres, Plow);
2. After full size-adjustment (age, height, weight, and BA) between the three groups (see Section 4.11.4).



No significant differences in mean BMC, BMD, or SA-BMC at the TH, FN, LS and WBLH were found between groups (Table 5-10). Unadjusted BA was significantly lower in Nref compared to Plow at the total hip (TH) (3.9 ±1.5%). At the lumbar spine (LS), Nref BA was significantly lower than Ppres and Plow (-3.7 ±1.4% and -4.2 ±1.4% respectively). Plow BA was significantly (-3.9 ±1.5%) lower than Ppres at whole body less head (WBLH). These differences in BA did not remain significant ($p>0.05$) when adjusted for age, height, and weight. Adjusting the BA values for stature and age removed the differences at TH and LS but not WBLH; however, after further adjustment for weight, these differences were no longer significant ($p>0.05$). There were no significant group differences in BMD SD-scores for any site measured ($p>0.05$) (Table 5-11).

Sequentially adding current cigarette smoking, current alcohol use, and personal history of bone fracture, or a combination of these into the models, did not alter the group comparisons of bone variables.

**Table 5-10 Total hip, femoral neck, lumbar spine, and whole body bone measures**

| | Nref *n*=98 Mean (SD) | Ppres *n*=74 Mean (SD) | Plow *n*=75 Mean (SD) | Group effect ANOVA P = |
|---|---|---|---|---|
| Total hip | | | | |
| BMC*(g) | 31.4 (5.3) | 31.2 (5.1) | 31.7 (4.8) | 0.84 |
| Area (cm$^2$) | 31.0 (3.08) | 31.7 (3.30) | 32.2 (3.18) [A] | 0.04 |
| BMD (g/cm$^2$) | 1.013 (0.131) | 0.985 (0.124) | 0.988 (0.125) | 0.30 |
| Femoral neck | | | | |
| BMC*(g) | 4.38 (0.59) | 4.38 (0.63) | 4.37 (0.66) | 0.99 |
| Area (cm$^2$) | 4.71 (0.34) | 4.79 (0.33) | 4.76 (0.37) | 0.31 |
| BMD (g/cm$^2$) | 0.930 (0.114) | 0.916 (0.125) | 0.923 (0.131) | 0.75 |
| Lumbar spine | | | | |
| BMC*(g) | 55.0 (9.0) | 57.5 (9.5) | 56.8 (9.4) | 0.21 |
| Area (cm$^2$) | 54.1 (4.84) | 56.1 (5.06) [A] | 56.3 (4.43) [A] | 0.003 |
| BMD (g/cm$^2$) | 1.018 (0.118) | 1.021 (0.109) | 1.006 (0.128) | 0.72 |
| WBLH | | | | |
| BMC*(g) | 1606 (232) | 1621 (241) | 1564 (224) | 0.30 |
| Area(cm$^2$) | 1670 (145) | 1714 (160) | 1647 (142) [B] | 0.02 |
| BMD(g/cm$^2$) | 0.958 (0.079) | 0.943 (0.071) | 0.947 (0.080) | 0.41 |

Area, bone area; BMC, bone mineral content; BMD, bone mineral density; SD, standard deviation; WBLH, whole body less head.
Letters are used to indicate significance of between-group differences as tested by ANOVA/Scheffé.
[A] Unadjusted value significantly different from Nref $P \leq 0.05$ but not significantly different after size adjustment.
[B] Unadjusted value significantly different from Ppres $P \leq 0.05$ but not significantly different after size adjustment. * No significant differences after full size adjustment (SA-BMC).

**Table 5-11 DXA BMD SDS relative to Nref[a]**

| | Ppres *n*=74 | N | Plow *n*=75 | N |
|---|---|---|---|---|
| Total hip | -0.211 (0.953) | 73 | -0.193 (0.953) | 74 |
| Femoral neck | -0.122 (1.096) | 73 | -0.063 (1.147) | 74 |
| Lumbar spine | 0.026 (0.923) | 73 | -0.099 (1.081) | 74 |
| WBLH | -0.194 (0.898) | 73 | -0.144 (1.009) | 73 |

All values are Mean ±SD.
DXA, dual-energy X-ray absorptiometry; SDS, standard deviation score.
WBLH, whole body less head (see Sections 4.4.1, 4.11.3).
[a] SDS for groups Ppres & Plow derived using Nref as the reference population.
There were no significant group differences.

## 5.7 Vitamin D status

Mean (SD) 25(OH)D for the whole cohort was 60.1 ±18.4 nmol/l and there were no significant differences between groups (p>0.05). 25(OH)D concentration was <50 nmol/l in 29.6% of individuals; with similar proportions in each of the groups in this category (26.5%, 29.7%, and 33.3% in Nref, Ppres, and Plow respectively). Very few subjects had a 25(OH)D concentration <25 nmol/l (1.0%, 2.7%, and 5.3% in the three groups respectively), despite the slightly greater number of Plow subjects whose blood samples for 25(OH)D measurement were obtained during the autumn and winter months (see Section 3.7) (Table 5-12).

**Table 5-12 Baseline vitamin D status**

| | Nref *n*=98 | Ppres *n*=74 | Plow *n*=75 | Group effect ANOVA P = |
|---|---|---|---|---|
| 25(OH)D (nmol/l) | 59.7 (16.5) | 59.2 (16.5) | 61.6 (22.3) | 0.69 |
| 25(OH)D (nmol/l) >50 (%) | 73.5 | 70.3 | 66.7 | |
| 25(OH)D (nmol/l) <50 (%) | 26.5 | 29.7 | 33.3 | |
| 25(OH)D (nmol/l) <25 (%) | 1.0 | 2.7 | 5.3 | |

There were no significant group differences.

### 5.7.1 Vitamin D status and fat mass

Fat mass did not predict 25(OH)D concentration in the group as a whole (n=247) at baseline; adjusted $R^2$ = 0.1%, coefficient -99.5x10$^{-6}$ (SE 271x10$^{-6}$), p=0.37. In ART-



exposed subjects the pattern was similar; adjusted $R^2$=-0.8%, coefficient 170.8x10$^{-6}$ (271.3x10$^{-6}$), p=0.53.

## 5.8 Biochemistry results

Serum phosphate was significantly higher in both the HIV-positive groups than HIV-negative women (p<0.0001) and higher in the Plow compared to Ppres (p=0.05). The magnitude of differences was: Ppres-Nref = 12.7 ±2.9%; Plow-Nref = 20.3 ±2.9% and Plow-Ppres = 7.6 ±3.1%.

TmP/GFR was significantly higher in the Ppres and Plow compared to Nref (11.2 ±3.2%; 27.4 ±3.2 % respectively) and higher in the Plow compared to Ppres 16.2 ±3.4%, (p=0.0002).

There were highly significant differences (p<0.005) in serum albumin at baseline between the groups with the HIV-negative women having significantly higher albumin concentrations that the HIV-positive women. Ppres and Plow were 6.5 ±1.3% and 12.3 ±1.3% lower than Nref respectively. Plow was significantly lower, 5.8 ±1.4%, than Ppres.

**Table 5-13 Baseline biochemistry**

| Analyte/ derived value | Nref *n*=98 | Ppres *n*=74 | Plow *n*=75 | Group effect ANOVA *P* = |
|---|---|---|---|---|
| Serum Cr (µmol/l) | 78.5 (20.8)[1] | 77.7 (17.7)[3] | 74.1 (22.1)[3] | 0.35 |
| eGFR | 81.6 (27.1)[1] | 78.9 (21.1)[3] | 87.6 (34.4)[3] | 0.16 |
| Serum albumin (g/l) | 40.1 (2.27)[1] | 37.6 (2.83)[A] | 35.6 (3.94)[A,B] | <0.0001 |
| Corrected calcium (mmol/l) | 2.20 (0.17) | 2.24 (0.13) | 2.20 (0.08) | 0.12 |
| Serum Pi (mmol/l) | 1.06 (0.16)[1] | 1.21 (0.24)[A] | 1.32 (0.34)[A,C] | <0.0001 |
| Serum ALP (U/l) | 61.3 (24.3)[1] | 57.4 (17.7) | 66.8 (27.3) | 0.05 |
| Urine P/Cr | 0.17 (0.40)[2] | 0.18 (0.27)[4] | 0.18 (0.36)[6] | 0.06 |
| TmP/GFR | 1.20 (0.27)[1] | 1.35 (0.34)[D,5] | 1.60 (0.49)[A,E,4] | <0.0001 |

All values are Mean (SD).
ALP, alkaline phosphatase; Cr, creatinine; eGFR, estimated glomerular filtration rate; P, phosphate; TmP/GFR, renal tubular maximum reabsorption rate of phosphate to glomerular filtration rate.

Letters are used to indicate significance of between-group differences as tested by ANOVA/Scheffé.
[A] Significantly different from Nref *P* ≤0.001.
[B] Significantly different from Ppres *P* ≤0.001.
[C] Significantly different from Ppres *P* ≤0.05.
[D] Significantly different from Nref *P* =0.001 - 0.01.
[E] Significantly different from Ppres *P* =0.001 - 0.01.
[1]n=97, [2] n=95, [3] n=73, [4] n=72, [5] n=71, [6] n=70

## 5.9 Factors influencing renal phosphate handling

The significance of the difference in serum phosphate results was not augmented by the addition of serum creatinine or eGFR into the linear models. Inclusion of age in the ANOVA model for serum phosphate resulted in a marginal decrease in the difference between Ppres and Plow (3.6 ±3.0%) (p=0.54). However, the differences between Nref and Ppres and Plow remained significant (p<0.05).

The significance of the difference in TmP/GFR was not augmented by the addition of serum creatinine or eGFR into the linear models.

## 5.10 Summary of groups differences at baseline

Nref and Ppres subjects were not different in any measure of body composition apart from WHR but both were different from Plow. Plow weighed significantly less (p≤0.02) and had significantly lower BMI (p≤0.002) than Nref and Ppres. Nref had significantly smaller WHR than Ppres and Plow (p≤0.01), suggesting a possible difference in fat



distribution phenotype in HIV-positive women associated with greater risk for cardio-metabolic disease, even before initiation of ART. Plow had significantly less body fat than Ppres or Nref (p≤0.001), and significantly less lean mass than Ppres (p=0.005). When expressed as fat:lean[2], which best described the relationship between fat and lean masses in all the women, Plow had significantly less fat per unit lean mass than Nref and Ppres (p≤0.02).

There were no significant differences in BMC, aBMD, or SA-BMC between the three groups. Unadjusted BA was significantly lower in Nref compared to Plow at the TH and in LS, and lower than Ppres at the LS. WBLH BA was significantly lower in Plow than Ppres (p<0.05). These differences in BA did not remain significant (p>0.05) when adjusted for age, height, and weight. Adjusting for current cigarette smoking, alcohol intake, and history of a previous fracture did not alter any differences in bone status between the groups. Examination of HIV-status, bone measures, and vitamin D status, revealed no significant correlations.

There were no significant differences in 25(OH)D between the three groups. There were highly significant differences in serum albumin concentrations (p≤0.005) with Nref>Ppres>Plow, and differences in serum phosphate and TmP/GFR (Nref<Ppres<Plow). Both Ppres and Plow had significantly higher serum phosphate than Nref.

The lack of difference in bone and vitamin D status between the groups, which is at variance from previously reported studies, may be the result of true lack of effect of HIV infection or reflect important differences in bone response to HIV between black Africans and Caucasians. The study design in which two distinct groups of HIV-positive women, based on South African eligibility criteria for ART-treatment, plus the inclusion of a HIV-negative control group strengthens the finding that HIV infection with varying degrees of immunosuppression does not appear to be driving alterations in BMD or vitamin D status in these young, urban women. The high rates of overweight may be masking an effect

**Table 5-13 Baseline biochemistry**

| Analyte/ derived value | Nref *n*=98 | Ppres *n*=74 | Plow *n*=75 | Group effect ANOVA *P* = |
|---|---|---|---|---|
| Serum Cr (µmol/l) | 78.5 (20.8)[1] | 77.7 (17.7)[3] | 74.1 (22.1)[3] | 0.35 |
| eGFR | 81.6 (27.1)[1] | 78.9 (21.1)[3] | 87.6 (34.4)[3] | 0.16 |
| Serum albumin (g/l) | 40.1 (2.27)[1] | 37.6 (2.83)[A] | 35.6 (3.94)[A,B] | <0.0001 |
| Corrected calcium (mmol/l) | 2.20 (0.17) | 2.24 (0.13) | 2.20 (0.08) | 0.12 |
| Serum Pi (mmol/l) | 1.06 (0.16)[1] | 1.21 (0.24)[A] | 1.32 (0.34)[A,C] | <0.0001 |
| Serum ALP (U/l) | 61.3 (24.3)[1] | 57.4 (17.7) | 66.8 (27.3) | 0.05 |
| Urine P/Cr | 0.17 (0.40)[2] | 0.18 (0.27)[4] | 0.18 (0.36)[6] | 0.06 |
| TmP/GFR | 1.20 (0.27)[1] | 1.35 (0.34)[D,5] | 1.60 (0.49)[A,E,4] | <0.0001 |

All values are Mean (SD).
ALP, alkaline phosphatase; Cr, creatinine; eGFR, estimated glomerular filtration rate; P, phosphate; TmP/GFR, renal tubular maximum reabsorption rate of phosphate to glomerular filtration rate.

Letters are used to indicate significance of between-group differences as tested by ANOVA/Scheffé.
[A] Significantly different from Nref $P \leq 0.001$.
[B] Significantly different from Ppres $P \leq 0.001$.
[C] Significantly different from Ppres $P \leq 0.05$.
[D] Significantly different from Nref $P = 0.001 - 0.01$.
[E] Significantly different from Ppres $P = 0.001 - 0.01$.
[1]n=97, [2] n=95, [3] n=73, [4] n=72, [5] n=71, [6] n=70

## 5.9 Factors influencing renal phosphate handling

The significance of the difference in serum phosphate results was not augmented by the addition of serum creatinine or eGFR into the linear models. Inclusion of age in the ANOVA model for serum phosphate resulted in a marginal decrease in the difference between Ppres and Plow (3.6 ±3.0%) (p=0.54). However, the differences between Nref and Ppres and Plow remained significant (p<0.05).

The significance of the difference in TmP/GFR was not augmented by the addition of serum creatinine or eGFR into the linear models.

## 5.10 Summary of groups differences at baseline

Nref and Ppres subjects were not different in any measure of body composition apart from WHR but both were different from Plow. Plow weighed significantly less (p≤0.02) and had significantly lower BMI (p≤0.002) than Nref and Ppres. Nref had significantly smaller WHR than Ppres and Plow (p≤0.01), suggesting a possible difference in fat



distribution phenotype in HIV-positive women associated with greater risk for cardio-metabolic disease, even before initiation of ART. Plow had significantly less body fat than Ppres or Nref (p≤0.001), and significantly less lean mass than Ppres (p=0.005). When expressed as fat:lean[2], which best described the relationship between fat and lean masses in all the women, Plow had significantly less fat per unit lean mass than Nref and Ppres (p≤0.02).

There were no significant differences in BMC, aBMD, or SA-BMC between the three groups. Unadjusted BA was significantly lower in Nref compared to Plow at the TH and in LS, and lower than Ppres at the LS. WBLH BA was significantly lower in Plow than Ppres (p<0.05). These differences in BA did not remain significant (p>0.05) when adjusted for age, height, and weight. Adjusting for current cigarette smoking, alcohol intake, and history of a previous fracture did not alter any differences in bone status between the groups. Examination of HIV-status, bone measures, and vitamin D status, revealed no significant correlations.

There were no significant differences in 25(OH)D between the three groups. There were highly significant differences in serum albumin concentrations (p≤0.005) with Nref>Ppres>Plow, and differences in serum phosphate and TmP/GFR (Nref<Ppres<Plow). Both Ppres and Plow had significantly higher serum phosphate than Nref.

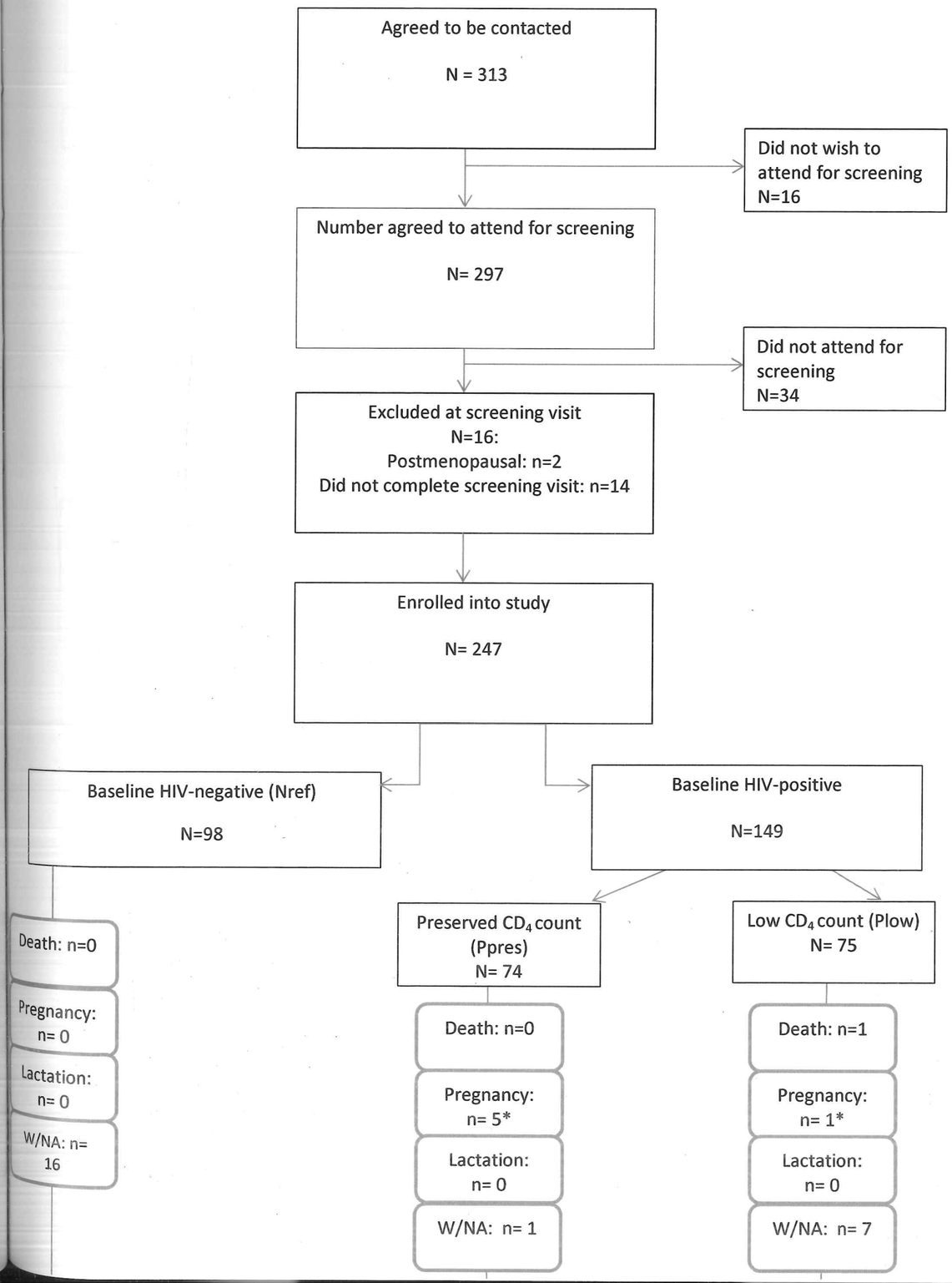
The lack of difference in bone and vitamin D status between the groups, which is at variance from previously reported studies, may be the result of true lack of effect of HIV infection or reflect important differences in bone response to HIV between black Africans and Caucasians. The study design in which two distinct groups of HIV-positive women, based on South African eligibility criteria for ART-treatment, plus the inclusion of a HIV-negative control group strengthens the finding that HIV infection with varying degrees of immunosuppression does not appear to be driving alterations in BMD or vitamin D status in these young, urban women. The high rates of overweight may be masking an effect

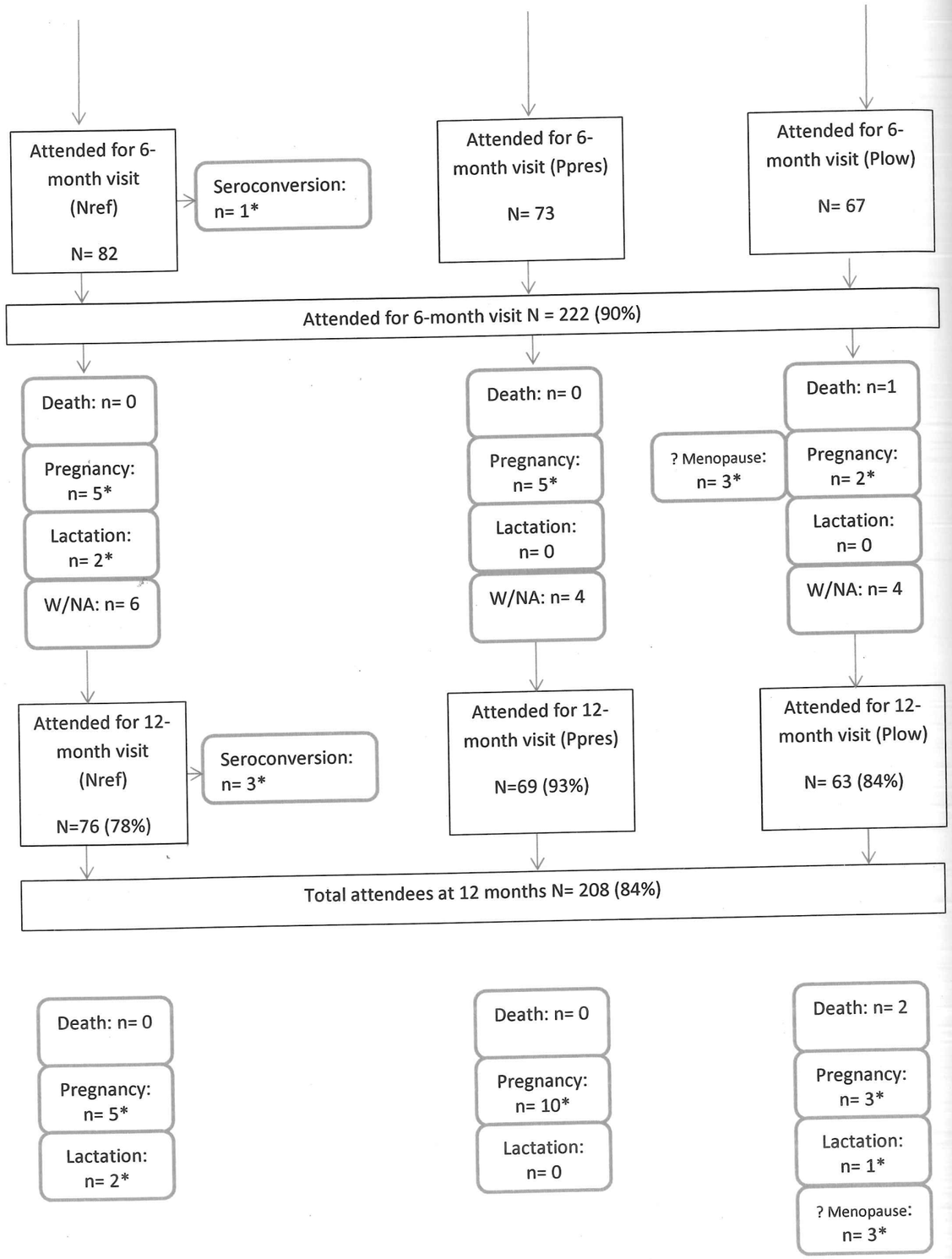
and more dramatic differences in aBMD and vitamin D might have been seen in women with advanced clinical HIV-disease not included in this study. The baseline data from this study provide an insight into bone health, body composition, and vitamin D status in African women living with HIV. They challenge the original hypotheses of this study, which was based on previously reported differences in aBMD and vitamin D status in HIV-positive subjects living in developed countries, and highlight the importance of studying subjects prior to ART exposure.



# 6 Follow-up results

## 6.1 Consort diagram for study






W/NA = withdrawal/non-attendance. *These participants remained in the study in the group assigned at baseline



### 6.1.1 HIV seroconversion and changes in $CD_4$ count

Nref women consented to HIV-testing at the six and 12 month study visits. The uptake of this was excellent with only one participant declining the test at six months. Over the course of the study, four (4.1%) participants seroconverted from HIV-negative to HIV-positive. This equates to 5.3% incidence rate (4/76) using the number of participants seen at 12 months as the denominator. One seroconversion occurred between baseline and 6 months, and three between the six and 12-month visits. Each woman was referred for confirmatory HIV-testing to the ZAZI clinic, PHRU and for $CD_4$ count measurement and entry into clinical care.

South African guidelines for initiating ART changed from a $CD_4$ count of ≤200 $x10^6$/l, to ≤350 $x10^6$/l during the course of the study. Some women in the Ppres group had falls in their $CD_4$ counts and were therefore initiated on ART, which had not been anticipated at the inception of the study to be likely before 12 months. Some women in the Plow group either had an increase in $CD_4$ or, more commonly, had not commenced ART as advised. There was therefore some switching between the predefined groups, according to $CD_4$ count status. Despite this, there were the predictable significant differences in $CD_4$ count between Ppres and Plow at the 6- and 12-month visits.

**Table 6-1 Changes in $CD_4$ counts from baseline**

| **Ppres** | 6 Months Mean (SD) N= 69 | % change from baseline (SE) | 12 Months Mean (SD) N= 64 | % change from baseline (SE) |
|---|---|---|---|---|
| $CD_4$ count $x10^6$ cells/l | 398 (116) | -4.8 (0.05) | 421 (163) | -2.6 (0.05) |
| Min | 99 | | 160 | |
| Max | 800 | | 1328 | |
| **Plow** | N=64 | | N= 62 | |
| $CD_4$ count x $10^6$ cells/l | 183 (114) | 11.6 (0.05) [A] | 256 (133) | 42.0 (0.05) [A] |
| Min | 18 | | 28 | |
| Max | 780 | | 600 | |

SE, standard error
Letters are used to indicate significance of between-group differences as tested by ANOVA/Scheffé.
[A] Significantly different from Ppres $P \leq 0.001$.

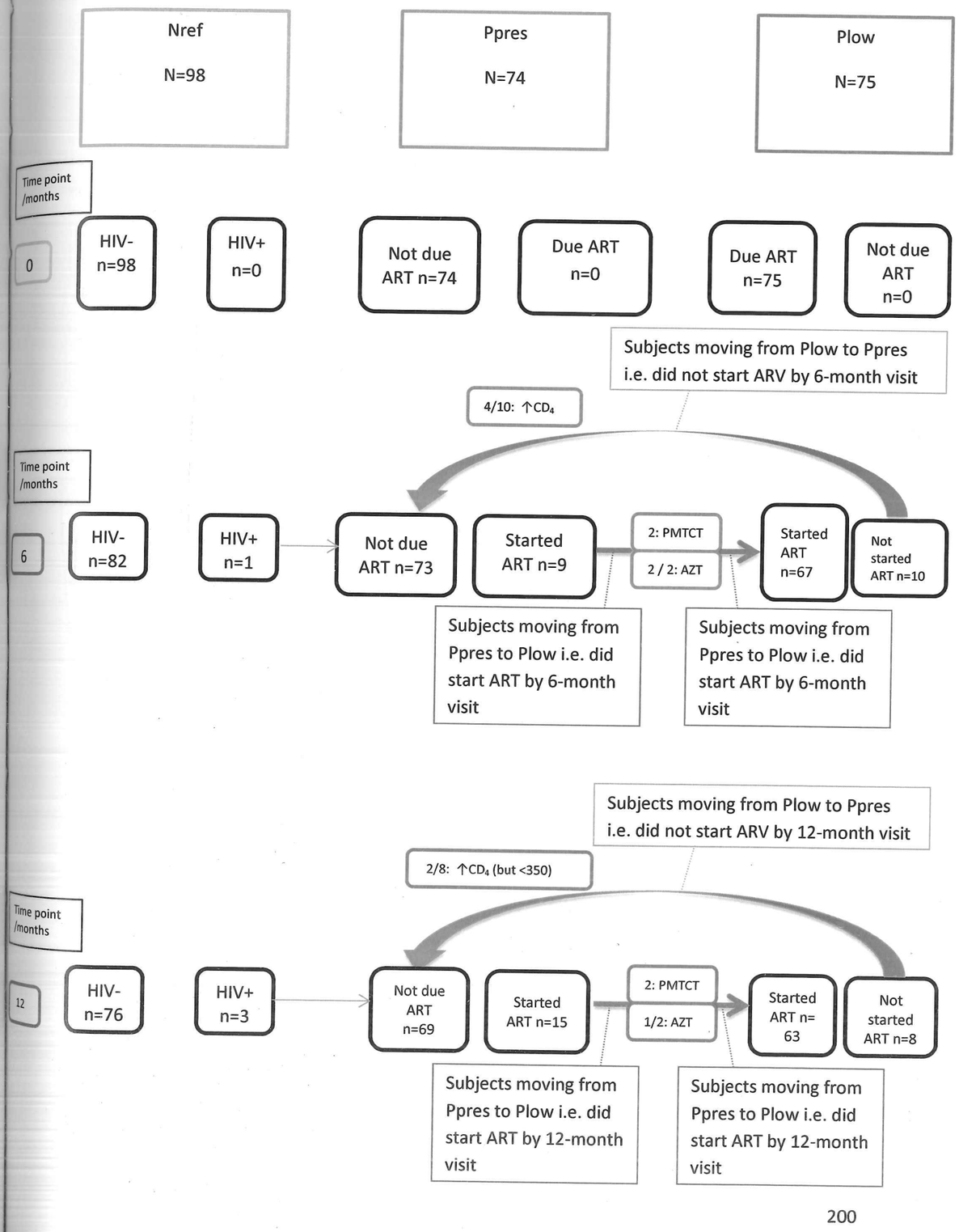
### 6.1.2 Group designation

As this was an observational study and not a clinical trial, a decision was made that data should be analysed based on the original group that subjects were assigned to. The numbers involved and the direction of movement are represented in Figure 6-1. As

previously discussed, a *post hoc* analysis of the data based on exposure to ART, was also performed; these data are presented in Chapter 8.



**Figure 6-1 Group changes**



## 6.2 6 month time point

### 6.2.1 Retention at 6 months

At the 6-month study visit, 10.1% (25/247) participants did not attend for their follow-up, resulting in data on 222 women being collected for analysis.

**Table 6-2 Losses to follow-up at 6 months**

| Group/ reason | Nref N (%) | Ppres N (%) | Plow N (%) |
|---|---|---|---|
| Loss to follow up | 17 (17.3) | 0 (0) | 8 (10.7) |
| Death | 0 (0) | 0 (0) | 1 (1.3) |
| No longer willing/able to take part | 2 (2.0) | 0 (0) | 0 (0) |
| Moved away | 1 (1.0) | 0 (0) | 0 (0) |
| Joined another study | 4 (4.1) | 0 (0) | 0 (0) |
| Not contactable | 10 (10.2) | 0 (0) | 7 (9.3) |

The reasons for non-attendance were death, no longer willing/able to take part, moved away, or joined another study and was ascertained in 32%; the remainder of participants were not contactable, despite several telephone calls and home visits where feasible (Table 6-2). Table 6-3 shows the mean (SD) duration from baseline to the 6-month visit for each of the three groups.

**Table 6-3 Mean (SD) duration of follow-up at 6-month visit**

| Group | N (%) | Baseline to 6-month visit days mean (SD) |
|---|---|---|
| Nref | 82 (84) | 173 (32) |
| Ppres | 73 (99) | 164 (26) |
| Plow | 67 (89) | 166 (25) |

There were no significant group differences.

Similarly, not all of the HIV-positive participants commenced ART at the same time after their baseline visit. Table 6-4 illustrates the mean duration of ART exposure.



**Table 6-4 Mean (SD) duration of ART exposure at 6-month visit**

| Group | N(%) | Duration of ART exposure* at 6-month visit days mean (SD) |
|---|---|---|
| Nref | NA | NA |
| Ppres | 9 (12) | 93 (93) |
| Plow | 50 (67) | 140 (39) |

NA, not applicable. * refers only to those who started ART.

The majority of patients who initiated ART were prescribed the South African standard, first line, triple combination of lamivudine, tenofovir, and efavirenz. Table 6-5 and Figure 6-2 describes the types of ARV drugs prescribed to Plow and Ppres women from baseline until they were reviewed at their 6 month visit. Figure 6-4 represents the proportions of different combination therapies in Plow and Ppres as a % of those exposed to ART in each group.

**Table 6-5 ART usage at 6-month visit**

| ART drug/Group | Ppres N (%) | Plow N (%) |
|---|---|---|
| AZT: Zidovudine | 2 (2.7) | 0 (0) |
| 3TC: Lamivudine | 7 (9.6) | 50 (74.6) |
| TDF: Tenofovir | 6 (8.2) | 48 (71.6) |
| D4T: Stavudine | 1 (1.4) | 1 (1.5) |
| ABC: Abacavir | 0 (0) | 0 (0) |
| NVP: Neviripine | 1 (1.4) | 6 (9.0) |
| EFV: Efavirenz | 6 (8.2) | 44 (65.7) |

**Figure 6-2 ART usage at 6-month visit**

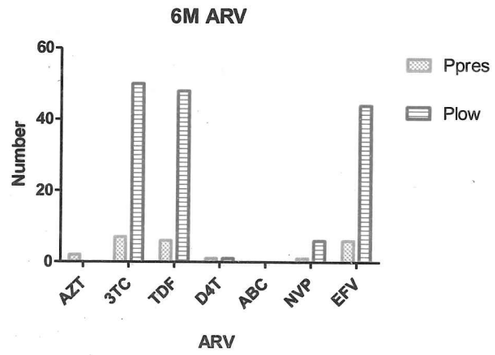
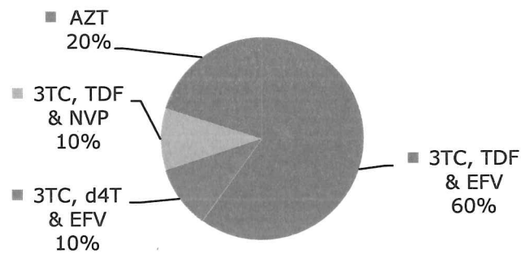
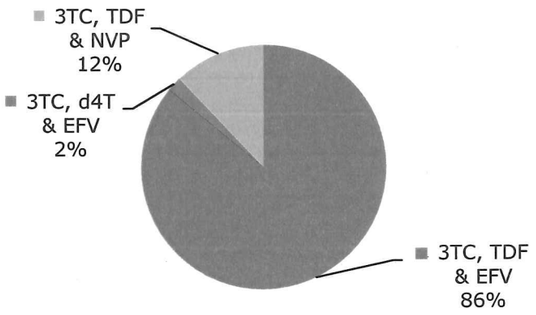


**Figure 6-3 ART combinations in Ppres at 6 months**




% of each combination of ART as total of those exposed to ART

**Figure 6-4 ART combinations in Plow at 6 months**




% of each combination of ART as total of those exposed to ART

### 6.2.2 Pregnancy

Figure 6-1 shows the number of pregnancies, after baseline and before the six month visit. There were 5 pregnancies (6.8%) in Ppres and 1 (1.5%) in Plow.

### 6.2.3 Anthropometry

At 6 months (Table 6-6) the mean weight in all groups had increased since baseline (Table 5-6) by 0.9, 0.3, and 1.8 kg (Nref, Ppres, and Plow respectively). Weight remained significantly less in Plow than Ppres and Nref. Weight in Ppres was non-significantly higher than in Nref. The median BMI was significantly lower in Plow than Nref and Ppres which were the same. Mean waist circumference was non-significantly greater in Nref and Ppres compared with Plow, and hip circumference was statistically lower in Plow compared to the other groups. There were non-significant differences in WHR (Table 6-6).



Fat mass remained significantly lower in Plow than Nref and Ppres. Lean mass was significantly lower in Plow than Ppres, and fat:lean[2] was significantly lower in Plow than Nref (Table 6-6).

**Table 6-6 Anthropometry at 6 months**

| | Nref *n*=82 | Ppres *n*=74 | Plow *n*=67 | Group effect ANOVA P = |
|---|---|---|---|---|
| Weight (kg) | 70.6 (17.1) | 72.3 (16.7)[1] | 64.1 (15.4)[A] | 0.01 |
| BMI (kg/m$^2$) median (IQR) | 27.4 (23.7;32.4) | 27.4 (24.2;32.3)[1] | 24.4 (21.4;27.9)[A,B] | 0.003 |
| >24.9 kg/m$^2$ (%) | 63 | 65 | 48 | |
| >30 kg/m$^2$ (%) | 34 | 38 | 18 | |
| <18.5 kg/m$^2$ (%) | 5 | 1 | 9 | |
| Waist circumference (cm) | 88.0 (15.2) | 88.0 (19.8)[2] | 83.4 (17.4) | 0.21 |
| Hip circumference (cm) | 109.0 (14.7) | 109.1 (13.7)[1] | 101.7 (12.6)[A,B] | 0.002 |
| Waist: Hip ratio | 0.81 (0.06) | 0.79 (0.17)[1] | 0.82 (0.12) | 0.52 |
| Fat mass (kg) | 26.6 (11.8) | 26.4 (10.1)[3] | 21.2 (9.1)[A,B,4] | 0.04 |
| Lean mass (kg) | 38.6 (5.7) | 39.9 (5.7)[3] | 36.9 (5.0)[A,4] | 0.01 |
| Fat:lean[2] (kg/kg$^2$) | 17.3 (4.7) | 16.3 (4.7)[3] | 15.1 (4.6)[B,4] | 0.02 |

Values are mean (SD).
Letters are used to indicate significance of between-group differences as tested by ANOVA/Scheffé.
[A] Significantly different from Ppres $P \leq 0.01$.
[B] Significantly different from Nref $P \leq 0.01$.
[1]n=72, [2]n=71, [3]n=67, [4]n=65

### 6.2.4 Bone measures

At the 6-month study visit, subjects had whole body DXA scans but no other skeletal sites because it was deemed unlikely to see changes in TH, FN, and LH after 6 months (see Section 4.4.1). There were significant differences in BA with Plow being significantly lower than Ppres, and in SA-BMC with Ppres significantly lower than Nref (Table 6-7).

**Table 6-7 Bone mineral status at 6 months**

| | Nref Mean (SD) *n*=82 | Ppres Mean (SD) *n*=66 | Plow Mean (SD) *n*=65 | Group effect ANOVA P = |
|---|---|---|---|---|
| **WBLH** | | | | |
| BMC (g) | 1608 (232) | 1637 (247) | 1561 (237) | 0.18 |
| BA (cm$^2$) | 1662 (140) | 1722 (165) | 1647 (146)[A] | 0.01 |
| BMD (g/cm$^2$) | 0.964 (0.082) | 0.947 (0.072) | 0.944 (0.085) | 0.29 |
| SA-BMC (g) | 1622 (111)[1] | 1574 (106)[B] | 1607 (125) | 0.04 |

Letters are used to indicate significance of between-group differences as tested by ANOVA/Scheffé.
[A] Significantly different from Ppres $P \leq 0.05$. [B] Significantly different from Nref $P \leq 0.05$. [1] n=81

### 6.2.5 Vitamin D

As at baseline, there were no mean differences between groups at 6 months (Table 6-8). However, all group mean 25(OH)D values were lower than at baseline, reflecting predictable decreases in vitamin D status during the winter and spring months.

**Table 6-8 Vitamin D status at 6 months**

| | Nref *n*=81 | Ppres *n*=73 | Plow *n*=67 | Group effect ANOVA P = |
|---|---|---|---|---|
| 25(OH)D (nmol/l) | 51.1 (48.8) | 50.4 (48.6) | 56.4 (21.5) | 0.12 |
| 25(OH)D (nmol/l) >50 % | 74 | 70 | 67 | |
| 25(OH)D (nmol/l) <50 % | 26 | 30 | 33 | |
| 25(OH)D (nmol/l) <25 % | 0 | 3 | 4 | |

All values are Mean ±SD unless stated.
25(OH)D, 25 hydroxyvitamin D. There were no significant group differences.

### 6.2.6 Biochemistry

At 6 months there were no significant differences in serum creatinine between groups by Scheffé *post hoc* testing, despite a significant group effect by ANOVA (p=0.02). As at baseline, there were significant differences in albumin across the groups with Nref being significantly higher than Ppres and Plow, and Ppres significantly higher than Plow. However, the difference between Plow and the other two groups had decreased over time. As at baseline, serum phosphate was significantly higher in Ppres and Plow compared to Nref at 6 months. TmP/GFR was significantly lower in Nref than Ppres and Plow at six months reflecting the differences at baseline.

There were increases in ALP activity in Plow compared to Ppres and Nref at the six-month timepoint. In Nref and Ppres the ALP activity was marginally lower at six months compared to baseline, whereas in Plow it had increased from a mean (SD) value of 66.8 (27.3) U/l to a mean of 80.0 (30.4) U/l. As this is a measure of total ALP activity it could suggest either hepatic or bone involvement. However, it is less common to see such large increases in hepatic ALP in response to ART treatment although this is possible. On balance, it probably suggests a skeletal origin for the rise in ALP in Plow, although this remains to be established. This difference in ALP was not materially affected when the few pregnant and lactating women were excluded from the analysis.



**Table 6-9 Biochemistry at 6 months**

| Analyte/ Derived variable | Nref *n*=82 | Ppres *n*=73 | Plow *n*=67 | Group effect ANOVA P = |
|---|---|---|---|---|
| Serum Cr (µmol/l) | 79.8 (12.4) | 74.0 (13.0)[4] | 77.7 (13.6) | 0.02 |
| eGFR | 75.4 (14.2)[1] | 81.8 (22.1)[4] | 77.4 (19.4) | 0.1 |
| Serum albumin (g/l) | 42.8 (2.9) | 39.6 (3.8)[A,5] | 38.7 (4.4)[A,B] | <0.0001 |
| Corrected calcium (mmol/l) | 2.40 (0.13) | 2.37 (0.15)[5] | 2.38 (0.14) | 0.39 |
| Serum P (mmol/l) | 1.15 (0.17) | 1.26 (0.24)[C,5] | 1.32 (0.29)[A] | 0.0001 |
| ALP (U/l) | 59.2 (21.0) | 52.4 (16.7)[5] | 80.0 (30.4)[A,B] | <0.0001 |
| Urine P/Cr | 1.22 (0.62)[2] | 1.23 (0.65)[6] | 1.13 (0.54)[7] | 0.53 |
| TmP/GFR | 1.23 (0.27)[3] | 1.40 (0.36)[C,4] | 1.49 (0.47)[A,8] | 0.0001 |

All values are Mean (SD).
ALP, alkaline phosphatase; Cr, creatinine; eGFR, estimated glomerular filtration rate; P, phosphate; TmP/GFR, renal tubular maximum reabsorption rate of phosphate to glomerular filtration rate.
Letters are used to indicate significance of between-group differences as tested by ANOVA/Scheffé.
[A] Significantly different from Nref $P \leq 0.001$.
[B] Significantly different from Ppres $P \leq 0.001$.
[C] Significantly different from Nref $P \leq 0.05$.
[1]n=81, [2]n=76, [3]n=78, [4]=n71, [5]n=72, [6]n=68, [7]n=63, [8]n=66

## 6.3 12-month time point

### 6.3.1 Retention at 12 months

At the 12-month study visit, 39/247 (15.8%) participants did not attend for their follow-up visit. The reason for non-attendance was ascertained in 9/39 (23.1%) at 12 months; the remainder of participants were not contactable, despite several telephone calls and home visits where feasible.

**Table 6-10 Losses to follow-up at 12 months**

| Reason/group | Nref | Ppres | Plow |
|---|---|---|---|
| Loss to follow up n (%) | 22 (22.4) | 5 (6.8) | 12 (16.0) |
| Death | 0 | 0 | 1 |
| No longer willing/able to take part | 4 | 0 | 2 |
| Moved away | 0 | 0 | 1 |

Table 6-11 shows the mean duration from baseline to the 12-month visit:

**Table 6-11 Mean (SD) duration of follow-up at 12-month visit**

| Group | N (%) | Baseline to 12-month visit days mean (SD) |
|---|---|---|
| Nref | 76 (76) | 358 (24) |
| Ppres | 69 (93) | 351 (25) |
| Plow | 63 (84) | 343 (21)[A] |

Letters are used to indicate significance of between-group differences as tested by ANOVA/Scheffé.
[A] Significantly different from Nref $P \leq 0.01$.

As participants commenced ART at different times in relation to their baseline visit the mean duration of ART usage was calculated at the 12-month visit. In Ppres 20% (n=15) initiated ART during the study period. An almost equal proportion in Plow due to commence ART did not (Table 6-12).

The vast majority of patients who initiated ART were prescribed the South African standard, first line, triple combination of lamivudine, tenofovir, and efavirenz (Table 6-13). At 12 months, one subject in Plow was treated with abacavir rather than tenofovir and this appeared to be because of concerns about her impaired renal function.

Figures 6-6 and 6-7 represent the proportions of different combination therapies in Plow and Ppres as a % of those exposed to ART in each group.

**Table 6-12 Mean (SD) duration of ART exposure at 12-month visit**

| Group | N (%) | Duration of ART exposure at 12-month visit days Mean (SD) |
|---|---|---|
| Nref | NA | NA |
| Ppres | 15 (20) | 177 (98) |
| Plow | 53 (71) | 293 (99) |



**Table 6-13 ART usage at 12-month visit**

| ART drug/ Group | Ppres N (%)* | Plow N (%) |
|---|---|---|
| AZT: Zidovudine | 0 (0) | 0 (0) |
| 3TC: Lamivudine | 14 (20.3) | 53 (84.1) |
| TDF: Tenofovir | 15 (21.7) | 52 (82.5) |
| D4T: Stavudine | 0 (0) | 0 (0) |
| ABC: Abacavir | 0 (0) | 1 (1.6) |
| NVP: Neviripine | 1 (1.4) | 6 (9.5) |
| EFV: Efavirenz | 14 (20.3) | 47 (74.6) |

*Information missing on use of 1 ART drug

**Figure 6-5 ART usage at 12-month visit**

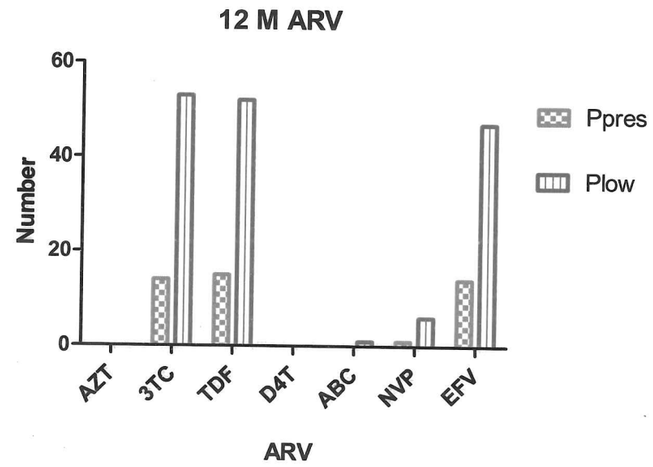
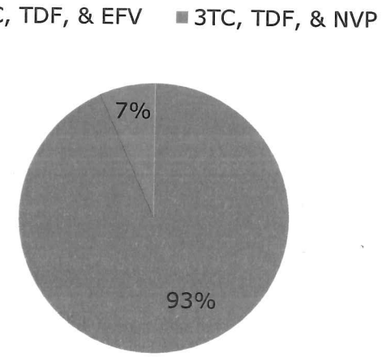
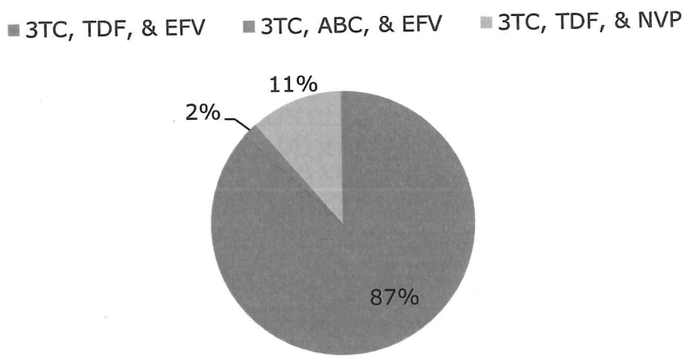



**Figure 6-6 ART combinations in Ppres at 12 months**




% of each combination of ART as total of those exposed to ART

**Figure 6-7 ART combinations in Plow at 12 months**




% of each combination of ART as total of those exposed to ART

### 6.3.2 Pregnancy, lactation, and menopause

As Figure 6.1 illustrates, 12 women became pregnant and 2 were lactating at the 12-month visit. Three women, all in Plow, ceased their regular menses between 6- and 12-month visits.

**Table 6-14 Pregnancy, lactation, and menopause at 12 months**

| Group | Nref N (%) | Ppres N (%) | Plow N (%) |
|---|---|---|---|
| Pregnancy | 5 (6.6) | 5 (7.2) | 2 (3.2) |
| Lactation | 0 | 2 (2.9) | 0 |
| Menopause | 0 | 0 | 3 (4.8)[†] |

[†]Possible menopause, last reported menses 3 – 6 months prior to visit

### 6.3.3 Anthropometry

At the 12-month visit the mean weights of Nref and Ppres were similar to each other and greater than Plow. Plow weighed 7kg less than Nref but this was no longer significant (p=0.06). BMI followed the same pattern with similar size effects, although Plow remained significantly lower than Nref. At 12 months Plow was still different in terms of weight from Nref and Ppres, which were similar to each other. Percentage overweight and obese (BMI >24.9 kg/m$^2$) were 73%, 65%, and 48% in Nref, Ppres, and Plow respectively. Waist circumference, waist:hip ratio, and lean mass were not significantly different between the groups. Hip circumference and fat mass were lower in Plow than Nref and Ppres, which were similar to each other. Fat mass was different between the groups (p=0.05), Plow was lower than Nref and Ppres by -2.4 ±1.9% and -1.9 ±1.9%



respectively. Fat:lean$^2$ were not significantly different between the groups, however Ppres and Plow were similar to each other and less than Nref (p=0.08).

The differences in anthropometry at baseline are still evident at 12 months.

**Table 6-15 Anthropometry at 12 months by original grouping**

| | Nref *n*=76 | Ppres *n*=69 | Plow *n*=63 | Group effect ANOVA P = |
|---|---|---|---|---|
| Weight (kg) | 73.0 (17.8)[1] | 71.0 (16.6) | 66.2 (15.3) | 0.06 |
| BMI (kg/m$^2$) median (IQR) | 28.2 (25.2;34.1)[1] | 27.4 (22.9;31.4) | 25.3 (21.4;29.1)[A] | 0.01 |
| >24.9 kg/m$^2$, (%) | 73 | 65 | 48 | |
| >30 kg/m$^2$ (%) | 34 | 35 | 19 | |
| <18.5 kg/m$^2$ (%) | 5 | 1 | 8 | |
| Waist circumference (cm) | 90.4 (15.1)[2] | 91.1 (16.1)[4] | 87.2 (13.6)[6] | 0.34 |
| Hip circumference (cm) | 110.8 (15.2)[1] | 107.9 (13.2)[4] | 104.4 (12.1)[A,6] | 0.03 |
| Waist:hip ratio (WHR) | 0.82 (0.07)[2] | 0.84 (0.12)[4] | 0.83 (0.06)[6] | 0.11 |
| WBLH fat (kg) | 28.4 (12.4)[3] | 25.6 (10.0)[5] | 23.7 (10.6)[B,6] | 0.05 |
| WBLH lean (kg) | 38.5 (5.9)[3] | 38.8 (5.6)[5] | 37.2 (3.4)[6] | 0.23 |
| Fat:lean$^2$ (kg/kg$^2$) | 18.5 (5.0)[3] | 16.8 (5.2)[5] | 16.8 (5.3)[6] | 0.08 |

All values are mean (SD) unless stated.
Letters are used to indicate significance of between-group differences as tested by ANOVA/Scheffé.
[A] Significantly different from Nref $P \leq 0.01$. [B] Non significantly different from Nref $P=0.055$.
[1]n=74, [2]n=73, [3]n=72, [4]n=68, [5]n=65, [6]n=62

### 6.3.4 Bone measures

At 12 months there were no significant group differences in BMC, BMD, and SA-BMC at any site. TH BA was significantly higher in Ppres (4.4 ±1.7%, p=0.03) and Plow (4.9 ±1.7%, p=0.02) than in Nref. LS BA was also significantly higher in Ppres (4.4 ±1.5%, p=0.03) and Plow (3.9 ±1.6%, p=0.04) than in Nref (Table 6-16). The differences in bone measures (i.e. BA) at baseline are still evident at 12 months. However, the effect of group on BA disappeared on size-adjustment. From baseline to 12 months there was a decrease in lumbar spine aBMD SDS (Z-score) from -0.099 (1.081) to -0.292 (1.101) in Plow, that is constistent with the changes demonstrated in Chapter 7.

**Table 6-16 Bone measures at 12 months**

| | Nref Mean (SD) *n*=72 | Ppres Mean (SD) *n*=65 | Plow Mean (SD) *n*=62 | Group effect ANOVA P = |
|---|---|---|---|---|
| **Total hip** | | | | |
| BMC (g) | 33.3 (6.3) | 33.8 (5.3) | 33.6 (5.6) | 0.89 |
| Area ($cm^2$) | 33.1 (3.1) | 33.5 (3.2)[A] | 33.7 (3.5)[A] | 0.006 |
| BMD ($g/cm^2$) | 1.036 (0.153) | 1.008 (0.130) | 0.995 (0.127) | 0.23 |
| SA-BMC (g) | 33.9 (4.4) | 33.3 (3.6) | 33.4 (4.0) | 0.70 |
| SDS | | -0.180 (0.853) | -0.265 (0.831) | |
| **Femoral neck** | | | | |
| BMC (g) | 4.43 (0.69) | 4.40 (0.67) | 4.33 (0.64) | 0.70 |
| Area ($cm^2$) | 4.78 (0.51) | 4.82 (0.32) | 4.82 (0.33) | 0.58 |
| BMD ($g/cm^2$) | 0.930 (0.139) | 0.914 (0.131) | 0.902 (0.133) | 0.47 |
| SA-BMC (g) | 4.39 (0.61) | 4.39 (0.51) | 4.39 (0.58) | 1.0 |
| SDS | | -0.113 (0.941) | -0.202 (0.958) | |
| **Lumbar spine** | | | | |
| BMC (g) | 55.0 (9.0) | 57.2 (9.4) | 55.1 (9.8) | 0.33 |
| Area ($cm^2$) | 53.4 (5.0) | 55.7 (5.0)[A] | 55.4 (4.6)[A] | 0.009 |
| BMD ($g/cm^2$) | 1.028 (0.125) | 1.022 (0.109) | 0.991 (0.138) | 0.20 |
| SA-BMC (g) | 55.5 (6.0) | 55.9 (5.3) | 54.7 (7.3) | 0.30 |
| SDS | | -0.046 (0.869) | -0.292 (1.101) | |
| **WBLH** | | | | |
| BMC (g) | 1614 (242) | 1625 (231) | 1574 (234) | 0.45 |
| Area ($cm^2$) | 1665 (141) | 1701 (145) | 1661 (146)[1] | 0.21 |
| BMD ($g/cm^2$) | 0.965 (0.086) | 0.952 (0.072) | 0.945 (0.081)[1] | 0.34 |
| SA-BMC (g) | 1624 (113) | 1591 (107) | 1599 (1220)[1] | 0.23 |
| SDS | | -0.150 (0.839) | -0.238 (0.945)[1] | |

SDS for groups Ppres & Plow derived using Nref as the reference population.
Letters are used to indicate significance of between-group differences as tested by ANOVA/Scheffé.
[A] Significantly different from Nref $P \leq 0.01$. [1]n=61

### 6.3.5 Vitamin D status

At 12 months there were no significant between-group differences in vitamin D status, the majority had 25(OH)D concentrations greater than 50 nmol/l (Table 6-17), and all group means were higher at 12 months compared to baseline and six months. The proportion with 25(OH)D concentrations <25nmol/l remained constant between groups over time suggesting that there was not a change in rates of those with lower vitamin D status.

**Table 6-17 Vitamin D status at 12 months**

| | Nref *n*=74 | Ppres *n*=68 | Plow *n*=62 | Group effect ANOVA P = |
|---|---|---|---|---|
| 25(OH)D (nmol/l) | 63.3 (17.7) | 66.0 (18.40) | 61.1 (20.1) | 0.32 |
| 25(OH)D >50 nmol/l, % | 73 | 71 | 66 | |
| 25(OH)D <50 nmol/l, % | 27 | 29 | 34 | |
| 25(OH)D <25 nmol/l, % | 0 | 3 | 5 | |

All values are Mean (SD) unless stated. 25(OH)D, 25 hydroxyvitamin D. There were no significant group differences.



### 6.3.6 Biochemistry

The 12-month biochemistry data are presented in Table 6-18. At 12 months there were no significant differences in serum creatinine between groups by Scheffé *post hoc* testing, despite a significant group effect by ANOVA (p=0.009). There were no significant between-group differences in eGFR, corrected calcium or urine phosphate:creatinine ratio. There were significant differences in serum albumin and serum phosphate. Nref albumin was significantly higher than Ppres or Plow, which were similar. Serum phosphate was significantly lower in Nref than Ppres and Plow which were similar. The serum albumin and phosphate differences are similar to those at baseline.

ALP activity was significantly higher in Plow than Nref and Ppres, which were similar, this also followed a similar pattern to baseline except the difference between Plow and Nref and Ppres were more pronounced (see Table 5-13). TmP/GFR was significantly lower in Nref than Ppres and Plow, which were similar to each other.

**Table 6-18 Biochemical analysis at 12 months**

| Analyte/ derived variable | Nref *n*=75 | Ppres *n*=69 | Plow *n*=63 | Group effect ANOVA P = |
|---|---|---|---|---|
| Serum Cr (µmol/l) | 77.3 (11.7)[1] | 73.3 (11.0)[3] | 74.9 (9.4) | 0.009 |
| eGFR | 78.1 (15.7)[1] | 80.9 (15.1)[3] | 78.7 (12.5) | 0.50 |
| Albumin (g/l) | 40.7 (2.9)[1] | 38.9 (3.4)[A,3] | 39.2 (3.6)[A] | 0.004 |
| Corr calcium(mmol/l) | 2.36 (0.12)[1] | 2.33 (0.13)[3] | 2.34 (0.15) | 0.39 |
| Serum P (mmol/l) | 1.12 (0.19)[1] | 1.28 (0.23)[B,3] | 1.32 (0.31)[B] | ≤0.0001 |
| ALP (U/l) | 71.1 (41.6)[1] | 66.7 (26.6)[3] | 90.0 (29.6)[A,C] | 0.0002 |
| Urine P/Cr | 1.64 (5.6) | 1.36 (0.7)[3] | 1. 47 (1.2)[4] | 0.89 |
| TmP/GFR | 1.19 (0.55)[2] | 1.38 (0.32)[A,3] | 1.45 (0.50)[B] | 0.004 |

All values are Mean (SD).
ALP, alkaline phosphatase; Corr, corrected; Cr, creatinine; eGFR, estimated glomerular filtration rate; P, phosphate; TmP/GFR, renal tubular maximum reabsorption rate of phosphate to glomerular filtration rate.

Letters are used to indicate significance of between-group differences as tested by ANOVA/Scheffé.
[A] Significantly different from Nref $P \leq 0.05$.
[B] Significantly different from Nref $P \leq 0.001$.
[C] Significantly different from Ppres $P \leq 0.001$.
[1]n=74, [2]n=72, [3]n=68, [4]n=62

## 6.4 Summary

The subject retention rate at 6 months was 90% and at 12 months was 84% (see Consort diagram, Section 6.1). Attrition at 12 months was 22.4%, 8.6%, and 16.0% in Nref, Ppres, and Plow respectively; the reasons were given in Table 6-10.

At six months, Plow were lighter than Nref and Ppres with lower BMI, waist, and hip circumferences, fat mass, lean mass, and fat:lean$^2$. As at baseline, Nref and Ppres were not different from each other at the six-month visit in any of these measures. The only significant difference in bone mineral status between the groups at six months was lower WBLH SA-BMC in Ppres compared with Nref, and a lower unadjusted BA in Plow than in Ppres. There were no differences in vitamin D status between the groups at six months. Serum albumin was significantly greater, and serum phosphate and TmP/GFR significantly less, in Nref compared to Ppres and Plow. These differences were in the same direction as at baseline. ALP activity had become significantly greater in Plow than Nref and Ppres.

At 12 months a similar pattern emerged with Nref and Ppres having greater weight, BMI, waist and hip circumferences, and fat and lean mass than Plow. At 12 months, Ppres was not different from Plow in fat:lean$^2$ but had a greater WHR ratio than both Nref and Plow. At 12 months there were no group differences in BMC, aBMD, or SA-BMC. There were significant differences in unadjusted BA at TH and LS with Nref being lower than Ppres and Plow at both sites. Measures of BMC and aBMD at 12 months were similar to those found at baseline, that is, no significant differences between the groups.

There were no significant differences in mean vitamin D status between the groups at 12 months. At this time point Nref had significantly higher serum albumin than Ppres and Plow and lower serum phosphate, ALP, and TmP/GFR, as at six months. At 12 months, as at other time points, Nref and Ppres characteristics were closer to each other than either to Plow.



A key observation is that Nref and Ppres were similar in most measures and more similar to each other than to Plow. It confirms the need to view HIV-positive individuals with advanced disease as distinct from those with preserved immune function who may be more physiologically and phenotypically similar to their HIV-negative counterparts.

# 7 Longitudinal modelling

## 7.1 Overview

This chapter explores longitudinal changes within individuals rather than comparing cross-sectionally the differences in groups at each of the three time points. In order to evaluate the changes in bone measures, body composition, and biochemical variables over time, hierarchical models were constructed wherein data for each individual could, where available, be used to determine individual change over time, 'nested' within the 3 groups assigned at baseline. Individual subjects were nested within their recruitment group and a time point-by-group interaction term derived (see Sections 4.11.4.5 and 4.11.4.6).

In order to best appreciate change over time, values were converted into natural logs, thereby allowing changes over time and differences between groups to be presented as sympercentages (see Section 4.11.2). All longitudinal values discussed as percentage change in the following chapters refer to sympercentages (mean (SE)). The absolute values for each variable at each time point were given in Chapters 5 and 6.

Table 7-1, Table 7-2, and Table 7-3 summarise the changes in body composition, bone and laboratory analysis from baseline to 12 months. The table is arranged to display differences by original grouping (Nref, Ppres, and Plow). Change was calculated from hierarchical linear models for each variable separately.

The relationship between the groups (Nref, Ppres or Plow) and the different time points (0, 6, and 12 months) was the main interaction assessed in order to determine whether the change over time differed by group. An interaction assesses whether the response, as measured by one of the variables, changes at different levels of the other factor (see Section 4.11.4.6).

In this section results are expressed in terms of percentage change from baseline as mean (SE), significances ≤0.05 are given. Scheffé *post hoc* test were used for individual pairs of timepoint and group to construct the graphs (see Section 4.11.4.3).



**Table 7-1 Anthropometry: percentage change (SE) from baseline at 12 months**

| Variable | Nref | Ppres | Plow |
|---|---|---|---|
| Weight (kg) | +2.3 (0.8) | -0.74 (0.8) | +3.9 (0.9) **<0.001** |
| BMI (kg/m$^2$) | +2.3 (1.1) | -1.1 (1.1) | +4.0 (1.2) **0.02** |
| Fat (kg) | +5.3 (1.7) **0.04** | -1.2 (1.8) | +10.2 (1.8) **<0.001** |
| Lean (kg) | +0.2 (0.5) | -1.7 (0.6) **0.05** | +0.3 (0.6) |
| Fat:lean$^2$ | +5.0 (1.7) | +2.3 (1.8) | +9.6 (1.9) **<0.001** |

Values are mean (SE) percentage change from baseline to 12 months in each group, *p*= in bold type. Where no *p* value stated, *p*>0.05.

**Table 7-2 Bone measures: percentage change (SE) from baseline at 12 months**

| Region/site | Nref | Ppres | Plow |
|---|---|---|---|
| Total hip | | | |
| BMC (g) | +6.1 (1.0) **<0.001** | +7.4 (1.1) **<0.001** | +4.7 (1.1) **<0.001** |
| BA (cm$^2$) | +3.6 (0.6) **<0.001** | +5.5 (0.7) **<0.001** | +4.3 (0.7) **<0.001** |
| BMD (g/cm$^2$) | +2.5 (0.5) **<0.001** | +1.9 (0.6) **0.004** | +0.4 (0.6) |
| SA-BMC (g) | +0.8 (0.5) | -0.3 (0.6) | -1.7 (0.6) **0.02** |
| Femoral neck | | | |
| BMC (g) | +1.6 (0.9) | -0.1 (0.9) | -0.8 (0.9) |
| BA (cm$^2$) | +1.5 (0.6) **0.03** | +0.5 (0.6) | +1.3 (0.6) |
| BMD (g/cm$^2$) | +0.6 (0.6) | -0.5 (0.6) | -2.4 (0.6) **0.001** |
| SA-BMC (g) | -0.3 (0.6) | -0.5 (0.6) | -2.7 (0.7) **<0.001** |
| Lumbar spine | | | |
| BMC (g) | +1.1 (0.6) | -1.0 (0.6) | -3.3 (0.7) **<0.001** |
| BA (cm$^2$) | -0.1 (0.3) | -0.8 (0.3) | -1.3 (0.3) **<0.001** |
| BMD (g/cm$^2$) | +1.2 (0.5) **0.05** | -0.2 (0.5) | -2.0 (0.6) **0.002** |
| SA-BMC (g) | +1.1 (0.5) | -1.1 (0.5) | -2.1 (0.6) **0.02** |
| WBLH | | | |
| BMC (g) | +0.2 (0.4) | -0.4 (0.4) | -1.0 (0.4) |
| BA (cm$^2$) | -0.6 (0.3) | -0.7 (0.3) | -0.3 (0.3) |
| BMD (g/cm$^2$) | +0.2 (0.3) | +0.6 (0.3) | -0.7 (0.3) |
| SA-BMC (g) | -0.3(0.3) | +0.5 (0.3) | -0.5 (0.3) |

Values are mean (SE) percentage change from baseline to 12 months in each group, *p*= in bold type. Where no *p* value stated, *p*.>0.05.

**Table 7-3 Vitamin D and biochemistry: percentage change (SE) from baseline to 12 months**

| Analyte/ derived variable | Nref | Ppres | Plow |
|---|---|---|---|
| 25(OH)D (mmol/l) | +6.6 (4.0) | +12.5 (4.2) | +2.0 (4.4) |
| Serum Cr (µmol/l) | +0.9 (2.9) | -4.4 (3.1) | +3.7 (3.2) |
| eGFR | -1.9 (3.4) | +4.5 (3.5) | -4.8 (3.7) |
| Albumin (g/l) | +1.5 (1.0) | +3.0 (1.0) | +9.1 (1.1) **<0.001** |
| Cor calcium (mmol/l) | +6.7 (0.9) **<0.001** | +3.8 (0.9) **0.002** | +5.8 (1.0) **<0.001** |
| Serum P (mmol/l) | +7.0 (2.3) **0.05** | +5.2 (2.4) | +0.0 (2.5) |
| ALP (U/l) | +13.1 ((3.5) **0.008** | +14.4 (3.7) **0.005** | +29.6 (3.8) **<0.001** |
| Urine P/Cr | +17.4 (11.5) | +10.3 (12.2) | +52.0 (12.7) **0.002** |
| TmP/GFR | +6.4 (3.3) | +3.8 (3.5) | -11.2 (3.6) **0.05** |

Values are mean (SE) percentage change from baseline to 12 months in each group, *p*= in bold type. Where no *p* value stated, *p*>0.05.
Cor calcium, corrected calcium; Cr, creatinine; P, phosphate.

Figure 7-1 to Figure 7-8 illustrate percentage changes in body composition, bone mineral, vitamin D status, and biochemical data from baseline. Nref is the reference value at baseline (timepoint 0) set at zero against which Ppres and Plow are set.

In the figures, Nref is represented in green, Ppres in orange and Plow in red. All data points are mean percentage change with error (SE) bars. The timepoint-by-group (Tp*Grp) interaction term is shown on each graph and describes the probability of a group difference in the relationship over time within individuals. All interaction terms described are timepoint-by-group interactions unless otherwise stated.

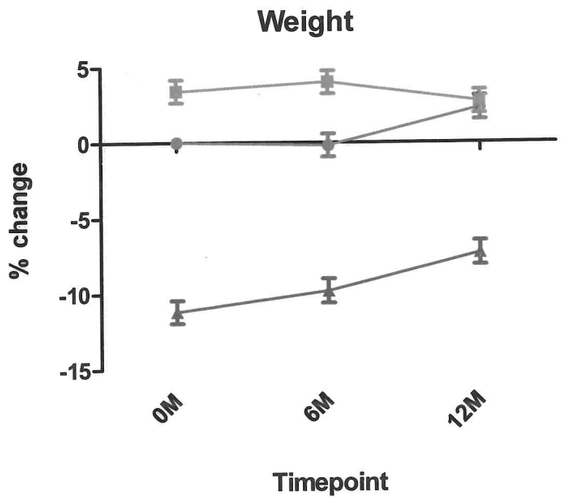
### 7.1.1 Anthropometric and body composition changes

At the 12-month visit the mean weight of Nref and Plow had increased (2.3 ±0.8% and 3.9 ±0.8% respectively), while it had fallen marginally in Ppres (-0.7 ±0.8%). Waist circumference had increased by 6.0 ±3.1% and 3.1 ±3.3% in Nref and Plow and decreased by 0.1 ±3.0% in Ppres. In contrast, hip circumference had increased most in Plow (4.1 ±1.7%), followed by Nref and Ppres (1.7 ±1.7% and 0.8 ±1.6% respectively). These changes in waist and hip circumferences translated into changes in waist:hip ratio (WHR) from baseline of 4.5 ±2.8%, -0.9 ±2.8%, and -1.0 ±2.9% in Nref, Ppres, and Plow respectively. There were changes in fat mass, significantly in Plow, Plow>Nref>Ppres of 10.2 ±1.8%, 5.3 ±1.7%, and -1.2 ±1.7% respectively. Lean mass also changed, significantly in Ppres, Ppres>Nref>Plow; -1.7 ±0.6%, 0.2 ±0.5%, and 0.3 ±0.6%. Fat:lean$^2$ increased in the same direction as fat mass with increases of 9.5 ±1.8%, 5.0 ±1.7%, and 2.2 ±1.7% respectively (Table 6-15). Figure 7-1 illustrates the within individual changes in body composition from baseline to 12 months. There was a significant increase in weight, BMI, and fat mass in Plow but not in Nref and Ppres (Tp*Grp p=0.0004, p=0.002, and p=0.004 respectively). Plow demonstrated a 10% increase in fat mass. The increases in fat mass in Plow were not mirrored by increases in lean mass. There was a 9.6% increase in fat:lean$^2$ in Plow subjects suggesting a preferential increase in fat rather than lean mass. In Ppres there was -1.7% change from baseline in lean mass (p=0.05), consistent with the -1.2% non-significant decrease in fat mass.

There were non-significant (p=>0.05) increases in waist and hip circumferences in Plow and in Nref and a non-significant decrease in waist and increase in hip circumference in Ppres. There were no significant changes in WHR from baseline to 12 months.

Plow saw significant increases of 10% in fat mass and fat:lean$^2$ and 4% increases in weight and BMI compared to Ppres, which demonstrated no significant change in these variables. Ppres had a significant decrease in lean mass over 12 months. The interaction terms for weight, BMI, and fat mass were significant confirming that the changes over time differ between groups. The interaction terms were not significant for waist and hip circumferences, WHR, lean mass and fat:lean$^2$.

Figure 7-1 Changes in body composition

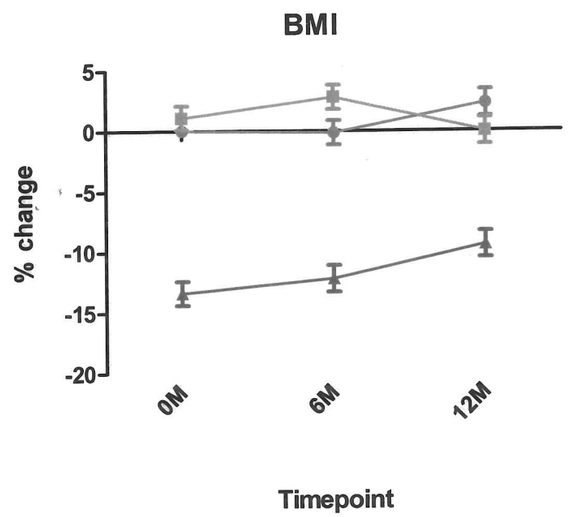



Timepoint*group interaction p=0.0004

Δ 2.3%, p=0.07

Δ -0.74%, p=0.94

Δ 3.9%, p=<0.001




Timepoint*group interaction p=0.002

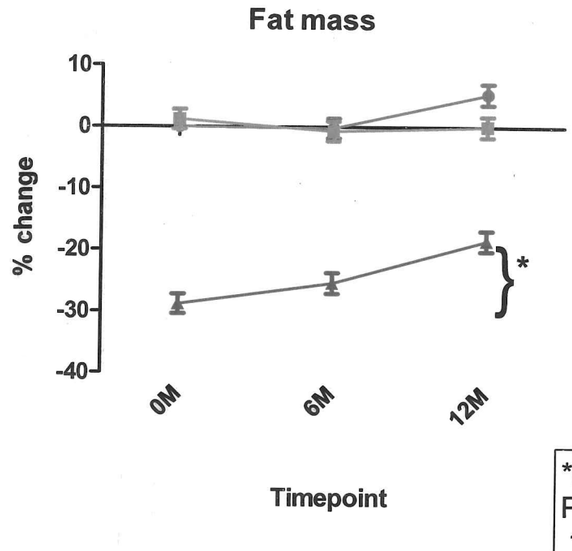
Δ 2.3%, p=0.34

Δ -1.1%, p=0.92

Δ 4.0%, p=0.02

Percentage changes from baseline to 12 months in weight and BMI (Nref in green, Ppres in orange and Plow in red). Nref at baseline is set at zero and all others are expressed relative to it.





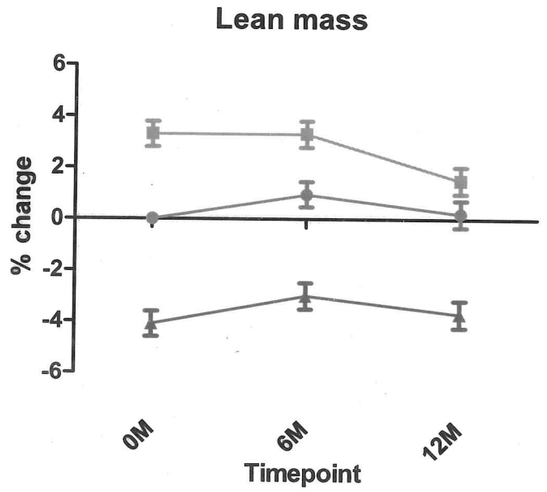

Timepoint*group interaction p=0.004

Δ 5.3%, p=0.04

Δ -1.2%, p=0.98

Δ 10.2%, p<0.001

*Difference between Plow at 0 and12 months 10.2%, p<0.001



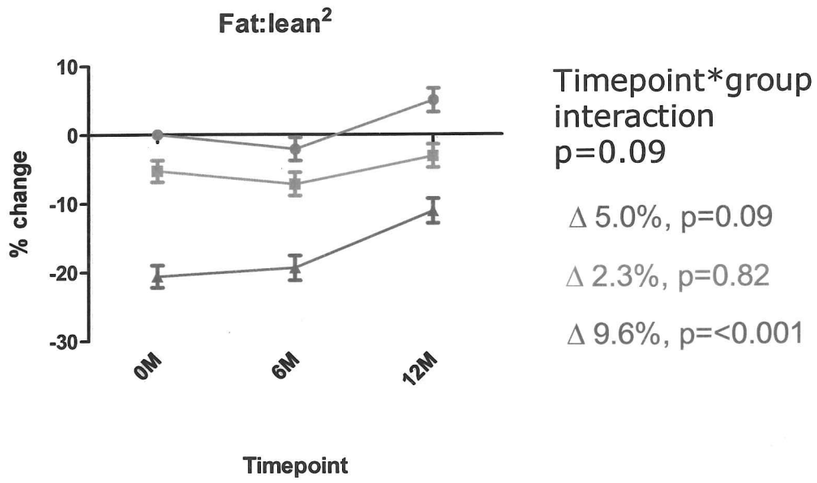

Timepoint*group interaction p=0.07

Δ 0.19%, p=1.0

Δ -1.7%, p=0.05

Δ 0.33%, p=0.99

Percentage changes from baseline to 12 months in fat mass and lean mass (Nref in green, Ppres in orange and Plow in red). Nref at baseline is set at zero and all others are expressed relative to it.




Timepoint*group interaction p=0.09

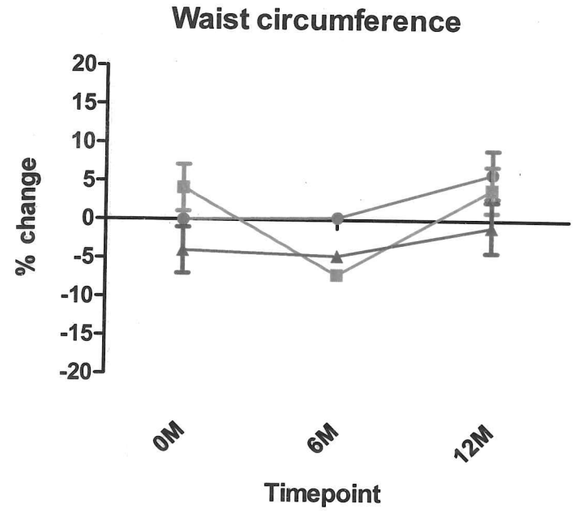
Δ 5.0%, p=0.09

Δ 2.3%, p=0.82

Δ 9.6%, p=<0.001

Percentage changes from baseline to 12 months in fat:lean² (Nref in green, Ppres in orange and Plow in red). Nref at baseline is set at zero and all others are expressed relative to it.



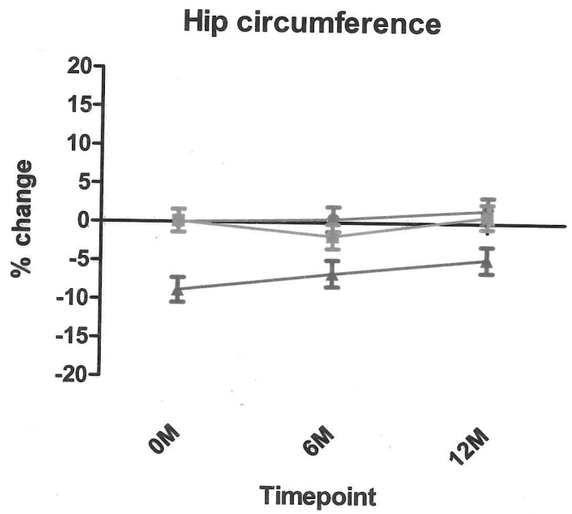



Timepoint*group interaction p=0.23

Δ 6.0%, p=0.45

Δ -0.14%, p=1.0

Δ 3.1%, p=0.93



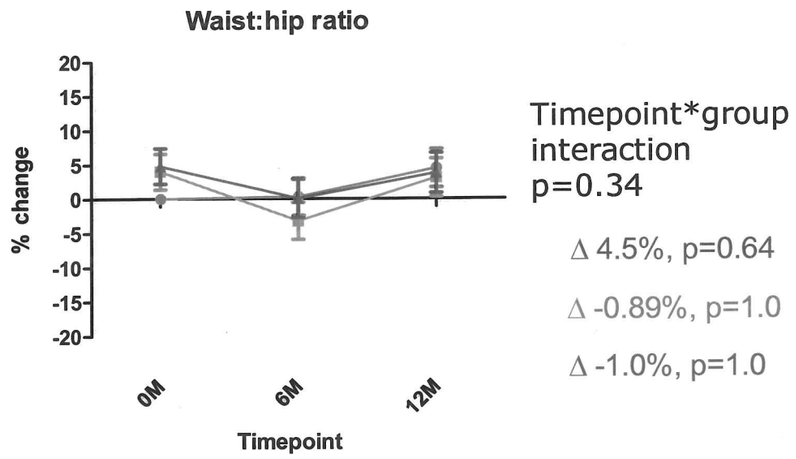

Timepoint*group interaction p=0.47

Δ 1.7%, p=1.0

Δ 0.74%, p=1.0

Δ 4.2%, p=0.27

Percentage changes from baseline to 12 months in waist circumference and hip circumference (Nref in green, Ppres in orange and Plow in red). Nref at baseline is set at zero and all others are expressed relative to it.




Percentage changes from baseline to 12 months in WHR (Nref in green, Ppres in orange and Plow in red). Nref at baseline is set at zero and all others are expressed relative to it.

### 7.1.2 Bone mineral status

Figure 7-2 to Figure 7-5 illustrate the within individual changes in bone measures from baseline to 12 months. These are expressed as percentage change from baseline to 12 months. The BMC data were adjusted for weight and BA to create SA-BMC (as height did not change over time). The absolute longitudinal changes in mean bone variables BMC, BA, BMD, and SA-BMC are also given in Table 7-2.

#### 7.1.2.1 Hip

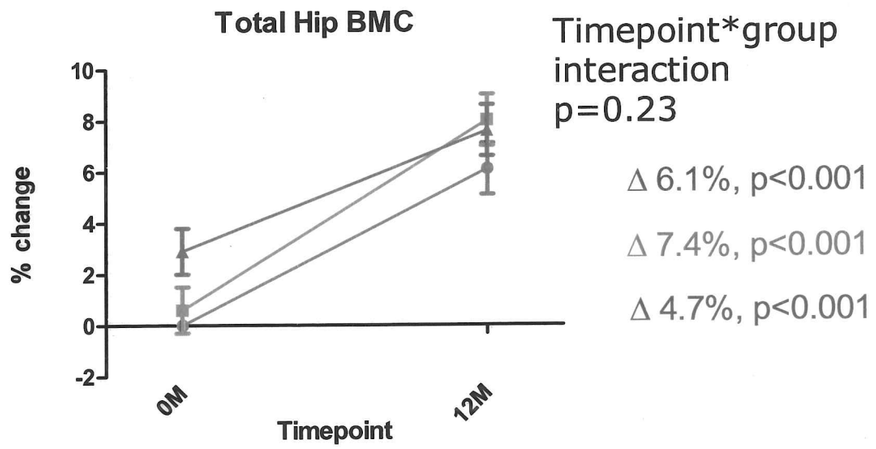
At the total hip there were increases in BMC and BA in all groups but with non-significant timepoint-by-group interactions, meaning that the changes over time within an individual were not significantly different from each other between the groups. The increases in hip BA were unexpected as bone expansion of this magnitude was unlikely in these adult women. It is probably a technical artefact, which is described in the discussion.

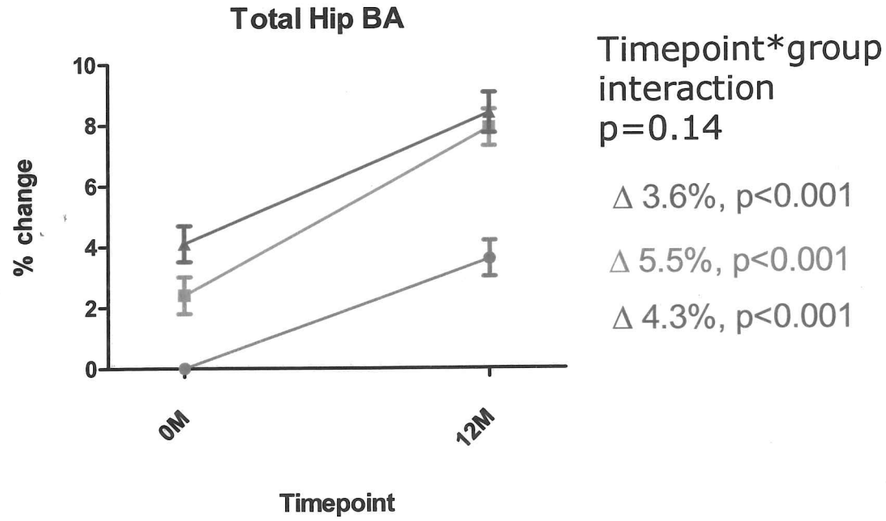
There were increases in hip aBMD in all groups, the largest increase was in Nref (2.5 ±0.5%, $p<0.01$) followed by Ppres 1.9 ±0.6% ($p=0.004$), and Plow 0.4 ±0.6% ($p=0.74$) with a significant interaction term ($p=0.03$). However, there was a significant decrease in hip SA-BMC of -1.7 ±0.6% ($p=0.02$) in the Plow group with a significant interaction term ($p=0.003$) and a 4.5% ($p<0.001$) difference between Nref and Plow



groups in SA-BMC (Figure 7-2). This showed that the bone mineral of Plow had not increased in proportion to their weight gain and increases in BA whereas for Nref the increases in BMC (and aBMD) were accounted for by their increased weight and BA.

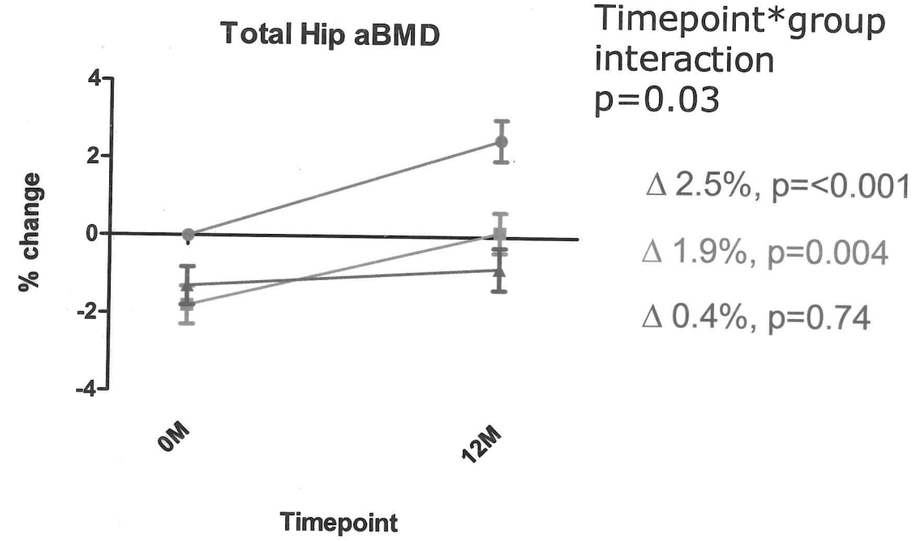
Figure 7-2 Changes in total hip

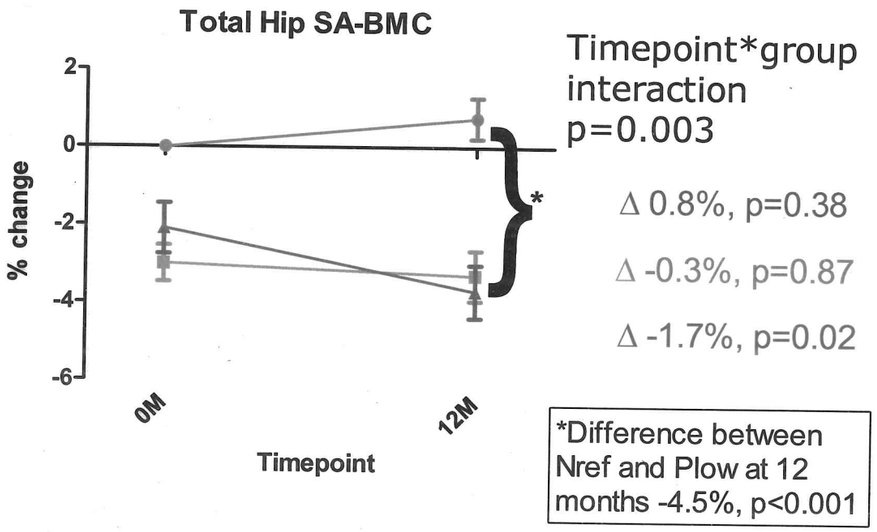



Percentage changes from baseline to 12 months in total hip bone measures (BMC & BA) (Nref in green, Ppres in orange and Plow in red). Nref at baseline is set at zero and all others are expressed relative to it.





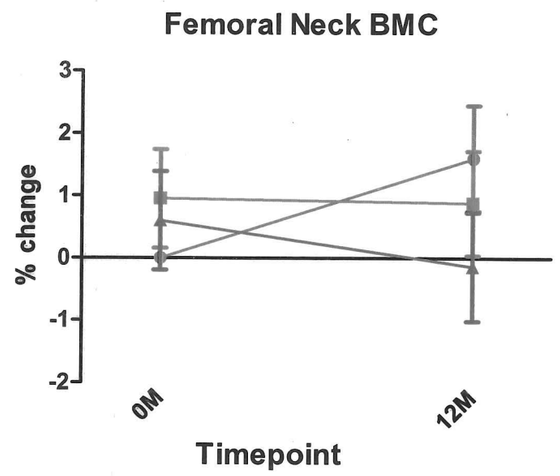

Percentage changes from baseline to 12 months in total hip bone measures (aBMD & SA-BMC) (Nref in green, Ppres in orange and Plow in red). Nref at baseline is set at zero and all others are expressed relative to it.

### 7.1.2.2 Femoral neck

At the femoral neck (FN) there were non-significant changes in BMC and BA in all groups, with the exception of BA in Nref (p=0.03). There was an increase in BMC in Nref and decrease in Ppres and Plow but the interaction term was not significant. aBMD decreased significantly in Plow -2.4 ±0.6% (p=0.001), but changed little in the other groups, with a significant interaction term (p=0.01). Similarly, there was a -2.7 ±0.7% (p<0.001) decrease in SA-BMC in Plow and a significant interaction term (p=0.01) resulting in a 1.8% (p=0.1) difference between Nref and Plow groups in SA-BMC at 12 months (Figure 7-3).



Figure 7-3 Changes in femoral neck

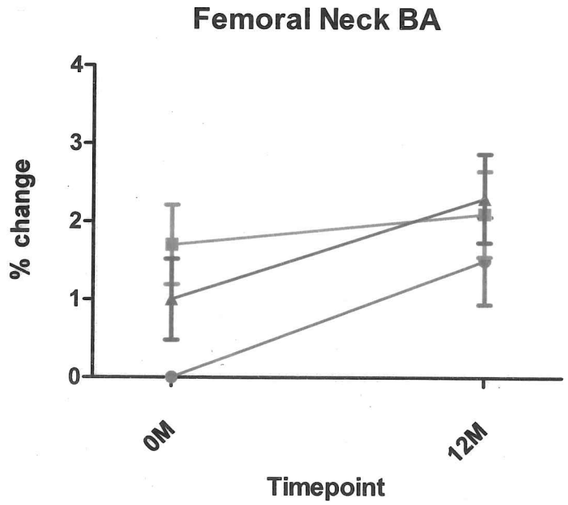



Timepoint*group interaction p=0.16

Δ 1.6%, p=0.18

Δ -0.1%, p=1.0

Δ -0.8%, p=0.71



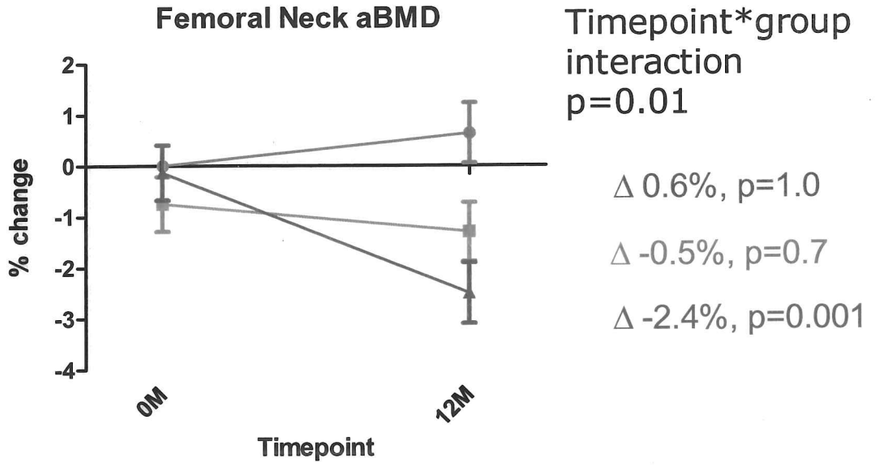

Timepoint*group interaction p=0.40

Δ 1.5%, p=0.03

Δ 0.5%, p=0.74

Δ 1.3%, p=0.10

Percentage changes from baseline to 12 months in femoral neck bone measures (BMC & BA) (Nref in green, Ppres in orange and Plow in red). Nref at baseline is set at zero and all others are expressed relative to it.

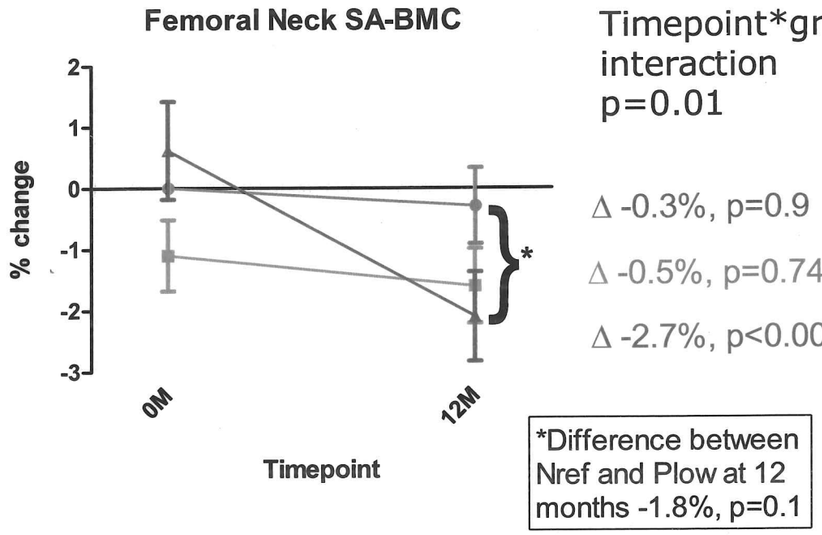



Timepoint*group interaction p=0.01

Δ 0.6%, p=1.0

Δ -0.5%, p=0.7

Δ -2.4%, p=0.001




Timepoint*group interaction p=0.01

Δ -0.3%, p=0.9

Δ -0.5%, p=0.74

Δ -2.7%, p<0.001

*Difference between Nref and Plow at 12 months -1.8%, p=0.1

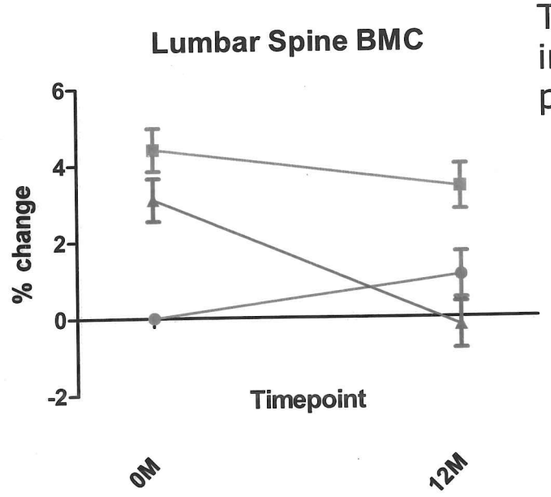
Percentage changes from baseline to 12 months in femoral neck bone measures (aBMD & SA-BMC) (Nref in green, Ppres in orange and Plow in red). Nref at baseline is set at zero and all others are expressed relative to it.



### 7.1.2.3 Lumbar spine

There were small, non-significant changes in BMC in Nref (1.1 ±0.6%) and Ppres (-1.0 ±0.6) at the lumbar spine. However, in the Plow group there was a -3.3 ±0.7% decline in BMC (p<0.001) with a significant interaction term p<0.0001. In BA there were small, non-significant changes in Nref (-0.1 ±0.3%) and Ppres (0.8 ±0.3%) but a significant decrease in Plow -1.3 ±0.3% (p=0.001) with a significant interaction term p=0.05. In aBMD there was a -2.0 ±0.6% (p=0.002) decrease in Plow and a 1.5 ±0.5% (p=0.05) increase in Nref with a significant interaction term (p=0.0001). There was a -2.1 ±0.6% (p=0.002) decrease in SA-BMC in Plow and a significant interaction term (p=0.0002) with non-significant changes in Nref and Ppres, resulting in a 4.3% (p<0.001) difference between Nref and Plow groups in SA-BMC (Figure 7-4).

Figure 7-4 Changes in lumbar spine

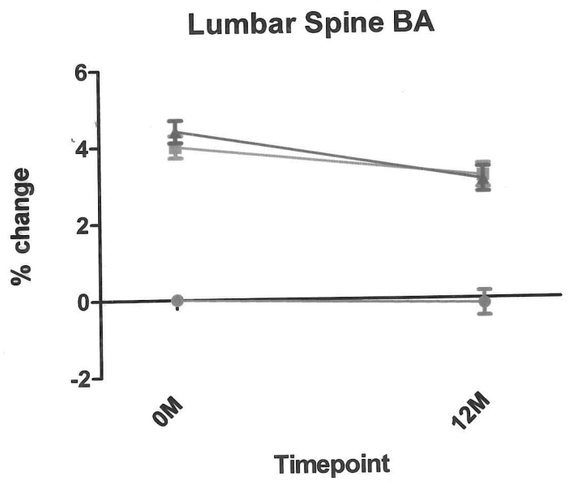



Timepoint*group interaction p=<0.0001

Δ 1.1%, p=0.18

Δ -1.0%, p=0.30

Δ -3.3%, p=<0.001




Timepoint*group interaction p=0.05

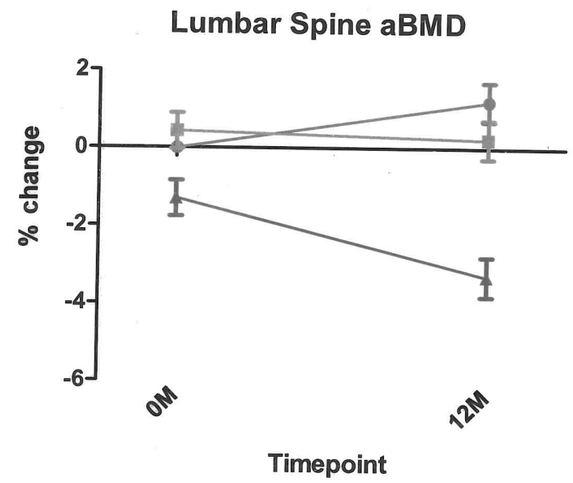
Δ -0.1%, p=0.9

Δ -0.8%, p=0.07

Δ -1.3%, p=0.001

Percentage changes from baseline to 12 months in lumbar spine bone measures (BMC & BA) (Nref in green, Ppres in orange and Plow in red). Nref at baseline is set at zero and all others are expressed relative to it.



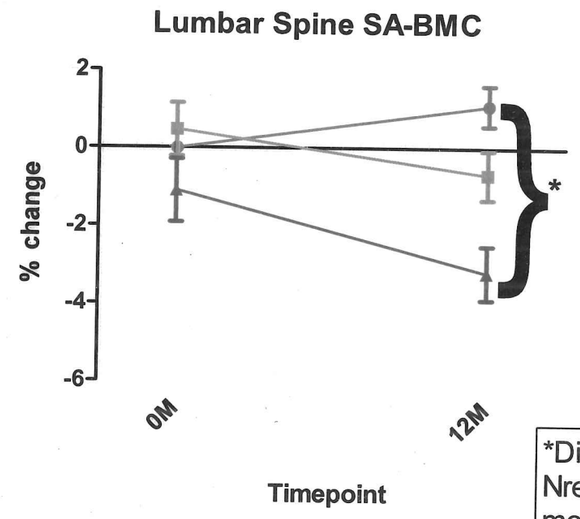



Timepoint*group interaction p=0.0001

Δ 1.2%, p=0.05

Δ -0.2%, p=0.93

Δ -2.0%, p=0.002




Timepoint*group interaction p=0.0002

Δ 1.1%, p=0.12

Δ -1.1%, p=0.98

Δ -2.1%, p=0.002

*Difference between Nref and Plow at 12 months -4.3%, p<0.001

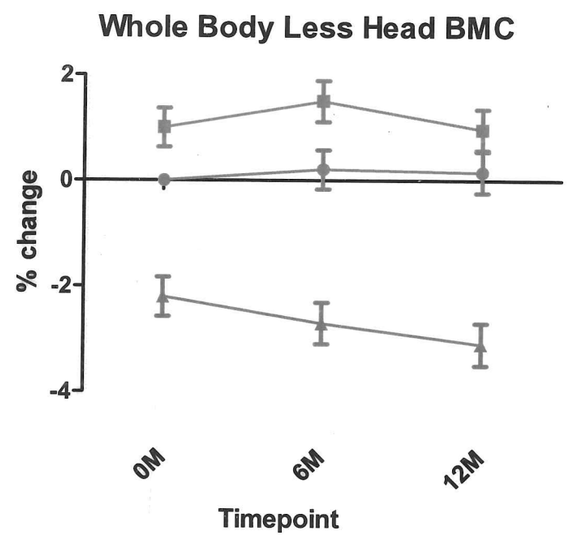
Percentage changes from baseline to 12 months in lumbar spine bone measures (aBMD & SA-BMC) (Nref in green, Ppres in orange and Plow in red). Nref at baseline is set at zero and all others are expressed relative to it.

### 7.1.2.4 Whole body less head

There were small, non-significant changes in BMC and BA in all groups. There were non-significant increases in WBLH aBMD and non-significant decrease in Plow but with a significant interaction term (p=0.03) showing that the changes were different between the groups. There were non-significant decreases SA-BMC in Nref and Plow and a non-significant increase in Ppres. At 12 months there was a -2.4% (p<0.001) difference between Nref and Plow groups in SA-BMC (Figure 7-5).



Figure 7-5 Changes in whole body

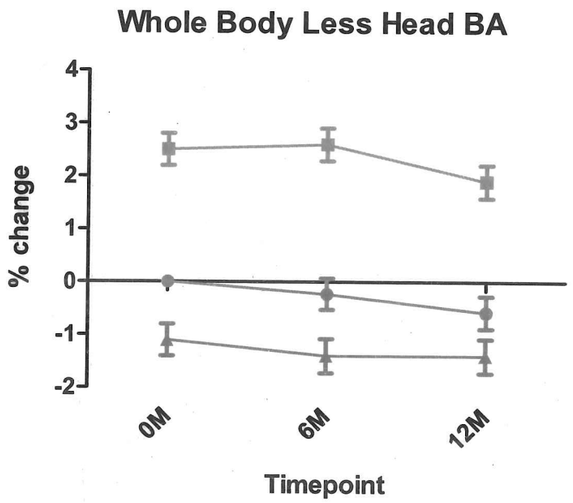



Timepoint*group interaction p=0.25

Δ 0.2%, p=1.0

Δ -0.4%, p=1.0

Δ -1.0%, p=0.29



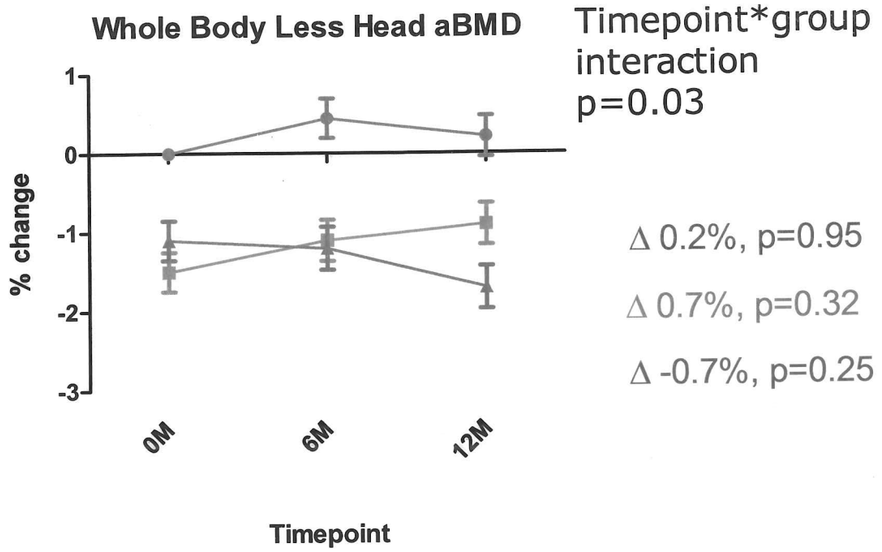

Timepoint*group interaction p=0.36

Δ -0.6%, p=1.0

Δ -0.7%, p=0.44

Δ -0.3%, p=0.95

Percentage changes from baseline to 12 months in whole body less head bone measures (BMC & BA) (Nref in green, Ppres in orange and Plow in red). Nref at baseline is set at zero and all others are expressed relative to it.

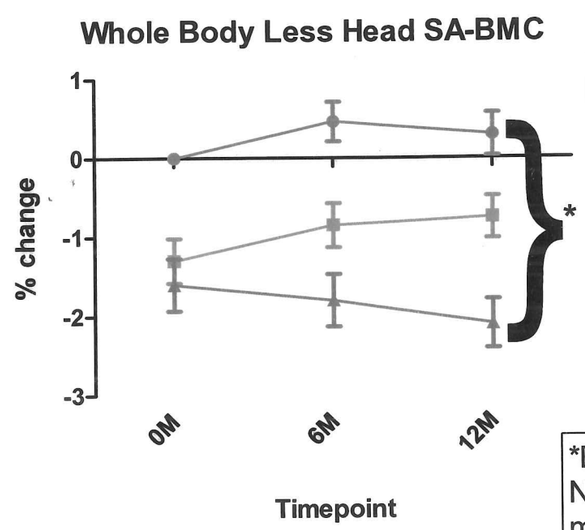



Timepoint*group interaction p=0.03

Δ 0.2%, p=0.95

Δ 0.7%, p=0.32

Δ -0.7%, p=0.25




Timepoint*group interaction p=0.10

Δ -0.3%, p=0.87

Δ 0.5%, p=0.45

Δ -0.5%, p=0.56

*Difference between Nref and Plow at 12 months -2.4%, p<0.001

Percentage changes from baseline to 12 months in whole body less head bone measures (aBMD & SA-BMC) (Nref in green, Ppres in orange and Plow in red). Nref at baseline is set at zero and all others are expressed relative to it.

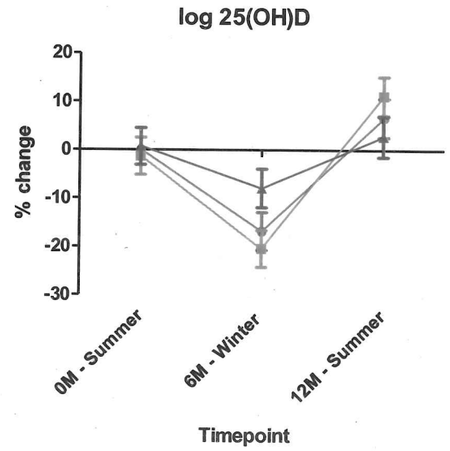


### 7.1.3 Changes in vitamin D status

The baseline visit (0) took place during the summer and autumn months (October – April) and the 12 month the following summer. By the 12-month visit serum 25(OH)D concentrations had not only increased from the 6-month (winter and spring, May – October) visit but exceeded baseline concentrations. There were 6.6 ±4.0%, 11.2 ±4.0%, and 2.8 ±4.2% increases from baseline in Nref, Ppres, and Plow respectively at 12 months (Table 6-17).

Figure 7-6 illustrates the within individual changes in vitamin D status from baseline to 12 months. There were significant, and predictable, decreases in mean winter and spring values from baseline in groups Nref, Ppres, and Plow of -16.8 ±3.9%, -20.4 ±3.8%, and -7.9 ±4.0% respectively. The winter decline in 25(OH)D concentrations in Ppres closely mapped that in Nref. Interestingly, the Plow group had a less steep winter decrease than Nref and Ppres. By 12 months there were non-significant increases from baseline but significant increases from six months.

**Figure 7-6 Changes in vitamin D status**




Timepoint*group interaction p=0.02

Δ 6.6%, p=0.6

Δ 12.5%, p=0.07

Δ 2.0%, p=0.99

Percentage changes from baseline to 12 months in 25(OH)D (Nref in green, Ppres in orange and Plow in red). Nref at baseline is set at zero and all others are expressed relative to it.

#### 7.1.3.1 Vitamin D and fat mass

To explore percentage changes in 25(OH)D and any relationship to adiposity the following model was constructed:

Y variable: log 25(OH)D at 12 months – log 25(OH)D at baseline

X variables:

- group
- log 25(OH) D at baseline (to prevent regression to the mean)
- log fat mass at 12 months – log fat mass at baseline (lnfat12-lnfat0)
- mean (log fat mass at 12 months + log fat mass at baseline)

Also, fat mass did not predict 25(OH)D concentration in the group as a whole (all groups) at six or 12 months. Change in fat did not predict change in vitamin D status. There were non-significant coefficients and interaction terms ($p>0.05$) at both time points. The group*lnfat12-lnfat0 interaction was non-significant, $p=0.5$.

### 7.1.4 Changes in biochemistry

At baseline there were significant differences between groups with Nref having significantly higher albumin concentrations than Ppres and Plow, and Ppres having significantly higher concentrations than Plow. Also, there were significant baseline differences in serum phosphate with Plow having significantly greater concentrations than Nref and Ppres, and Ppres significantly higher than Nref. These serum phosphate differences were reflected in TmP/GFR (see Table 5-13).

At 6 months compared to baseline (Table 5-13) there were significant increases in serum albumin in all groups; 6.7 ±0.94%, 5.2 ±0.94%, and 8.6 ±0.98% in Nref, Ppres, and Plow respectively. There were increases in serum albumin concentration from baseline in Plow, 9.1 ±1.1% ($p<0.001$) and non-significant increases in Nref and Plow over 12 months. There were changes in ALP activity from baseline to 6 months; an increase in Plow by 15.5 ±3.6% compared with a fall of 8.4 ±3.5% in Ppres (see Table 6-9).

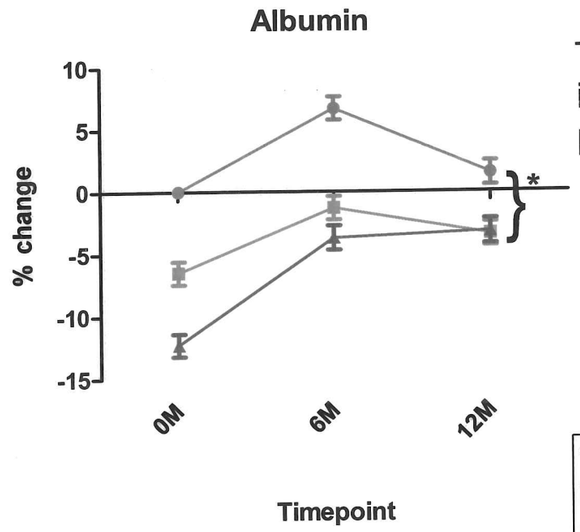


There were significant increases in corrected calcium in all groups but a non-significant interaction term ($p>0.05$). Serum creatinine concentrations remained stable in all groups (between +3.7% to -4.4%, $p>0.05$). Predictably, changes in eGFR mirrored changes in serum creatinine and were non-significant.

There were further increases in albumin in Plow (0.5 ±1.0%) from 6 to 12 months, a decrease in Ppres (-2.1 ±0.96%), and larger decreases in Nref (-5.2 ±0.97%). The increases in albumin in Plow probably reflect improvements in nutritional status and decreased catabolism after the initiation of ART. There was a further 14.1 (3.6)% increase in ALP activity from 6 months to 12 in Plow. There were increases in serum phosphate in Nref. In Plow there was a decline in TmP/GFR of 11.2% over the study period although serum phosphate remained constant. This possibly reflected increases in urinary phosphate loss with liberation of phosphate from bone in order to maintain serum concentrations and is supported by the markedly raised ALP activity (Table 6-18). From baseline there were large, significant increases in ALP activity in all groups, with the largest increases seen in Plow at 29.6 ±3.8% ($p<0.001$) at 12 months.

Figure 7-7 illustrates the within individual changes in biochemistry from baseline to 12 months.

Figure 7-7 Changes in biochemistry and derived variables



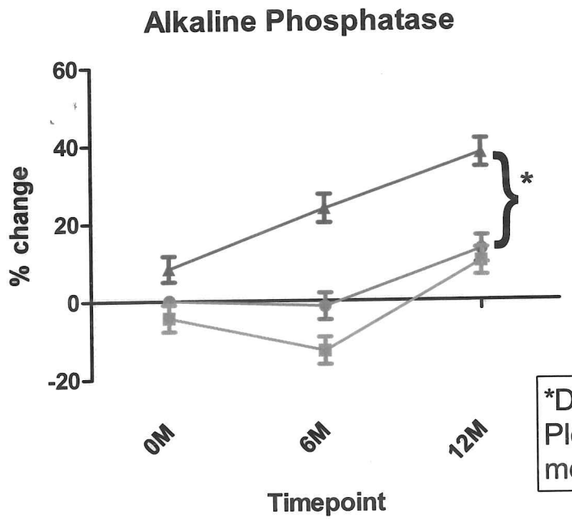

Timepoint*group interaction p=<0.0001

Δ 1.5%, p=0.67

Δ 3.0%, p=0.07

Δ 9.1%, p=<0.001

*Difference between Plow and Nref at 12 months -4.7%, p<0.001




Timepoint*group interaction p=<0.001

Δ 13.1%, p=0.008

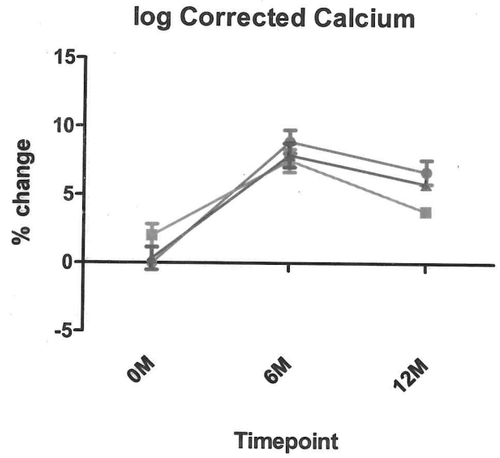
Δ 14.4%, p=0.005

Δ 29.6%, p=<0.001

*Difference between Plow and Nref at 12 months 24.8%, p<0.001

Percentage changes from baseline to 12 months in biochemical analytes and derived variables (Nref in green, Ppres in orange and Plow in red). Nref at baseline is set at zero and all others are expressed relative to it.



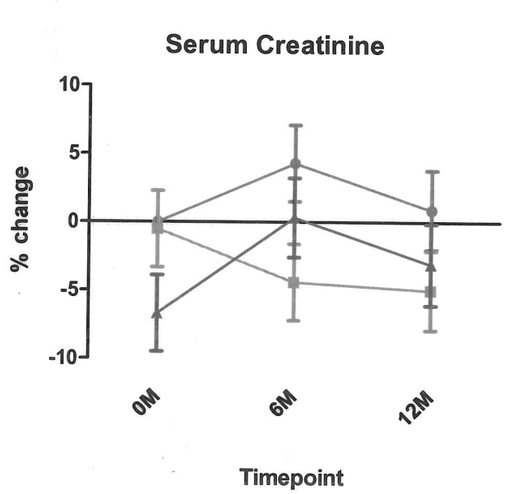



Timepoint*group interaction p=0.07

Δ 6.7%, p=<0.001

Δ 3.8%, p=0.002

Δ 5.8%, p=<0.001



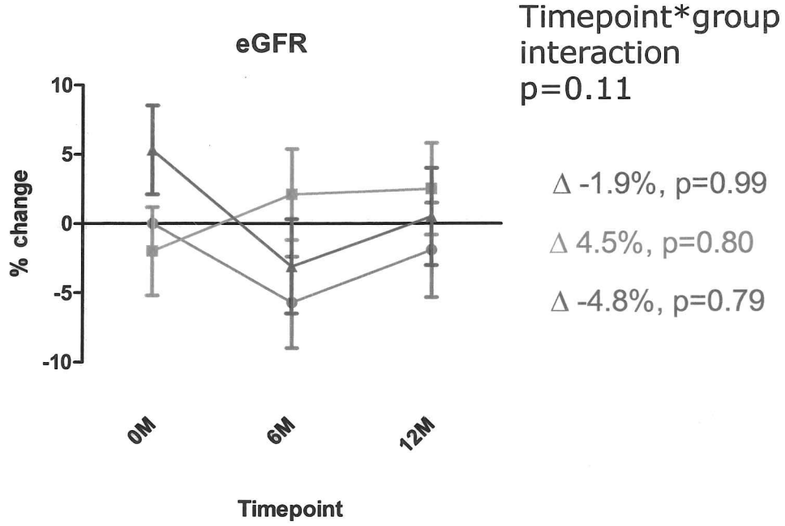

Timepoint*group interaction p=0.11

Δ 0.9%, p=1.0

Δ -4.4%, p=0.72

Δ 3.7%, p=0.90

Percentage changes from baseline to 12 months in biochemical analytes and derived variables (Nref in green, Ppres in orange and Plow in red). Nref at baseline is set at zero and all others are expressed relative to it.




Timepoint*group interaction p=0.11

Δ -1.9%, p=0.99

Δ 4.5%, p=0.80

Δ -4.8%, p=0.79

Percentage changes from baseline to 12 months in biochemical analytes and derived variables (Nref in green, Ppres in orange and Plow in red). Nref at baseline is set at zero and all others are expressed relative to it.

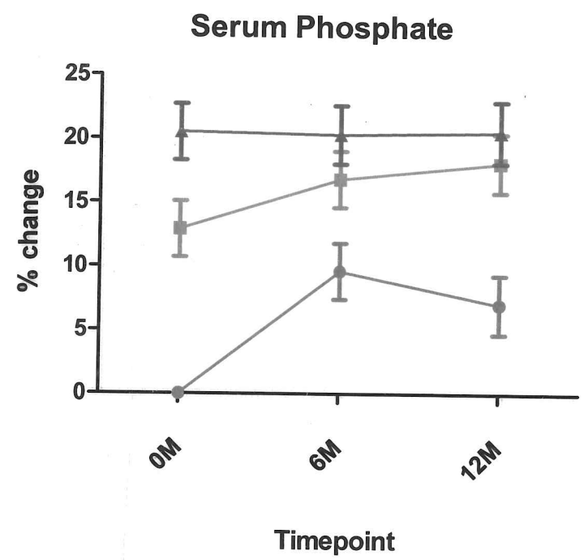
### 7.1.5 Changes in markers of phosphate homeostasis

At baseline, Plow had significantly higher mean serum phosphate concentrations than Nref or Ppres ($p \leq 0.05$) and TmP/GFR was significantly higher in Plow, (see Section 5.8). By six months, serum phosphate increased in Nref and Ppres (by 9.6 ±2.2% and 3.9 ±2.2% respectively) and remained stable in Plow.

At 12 months there was an overall increase in serum phosphate in Nref and Ppres; 7.0 ±2.3% (p=0.05), and 5.2 ±2.4% (p=ns) respectively. There was no change in Plow 0.0 ±2.5% (p=ns). At six months TmP/GFR increased in Nref and Ppres by 4.6 ±3.2% and 4.4 ±3.5% respectively but declined by 7.3 ±3.6% in Plow. By 12 months there were non-significant increases in Nref and Ppres of 6.4 ±3.3% (p=0.45) and 3.8 ±3.5% (p=0.88), which was consistent with serum phosphate change. There was a significant decrease in TmP/GFR in Plow, -11.2 ±3.6% (p=0.05), which was consistent with changes in patterns of serum phosphate. At 12 months there was a 9.9 ±3.8% (p=0.14) difference in TmP/GFR between Nref and Plow. Figure 7-8 illustrates the within individual changes in phosphate handling from baseline to 12 months.



Figure 7-8 Changes in phosphate metabolism

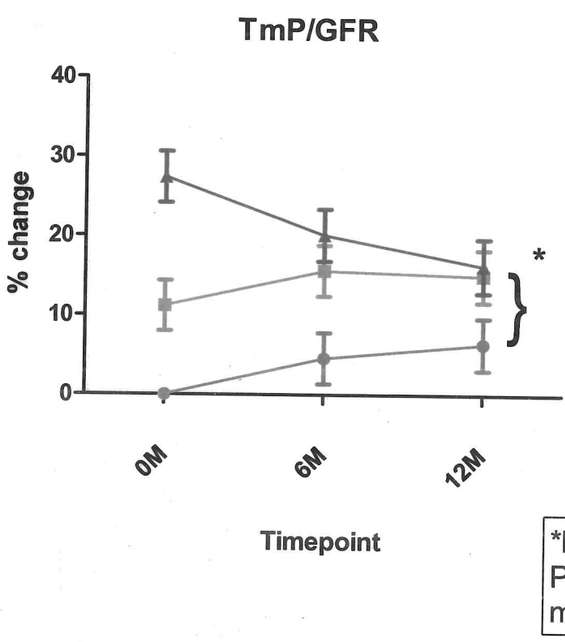



Timepoint*group interaction p=0.04

Δ 7.0%, p=0.05

Δ 5.2%, p=0.32

Δ 0.002%, p=1.0




Timepoint*group interaction p=0.004

Δ 6.4%, p=0.45

Δ 3.8%, p=0.88

Δ -11.2%, p=0.05

*Difference between Plow and Nref at 12 months 9.9%, p=0.14

Percentage changes from baseline to 12 months in markers of phosphate metabolism (Nref in green, Ppres in orange and Plow in red). Nref at baseline is set at zero and all others are expressed relative to it.

## 7.2 Summary

Longitudinal analysis of these data, using hierarchical models, provides a more detailed exploration of change within individuals over 12 months, and allows for an exploration of differences between groups in change over time that were not evident following cross-sectional analyses.

These analyses reveal a 3.9 ±0.9% increase in weight and a 10.2 ±1.8% increase in fat mass in Plow. Nref also gained weight and fat mass but half the amount compared with Plow. Ppres had decreases in weight, BMI, fat mass and lean mass over the course of the study. The large increases in weight and fat mass in Plow are important as they could indicate an increased risk of future metabolic disease such as impaired glucose tolerance and insulin resistance.

There were significant increases in hip BMC in all groups ($p<0.001$) but no significant changes in femoral neck BMC. At the lumbar spine there was a 3.3 ±0.7% decrease in BMC in Plow with no significant changes in Nref or Ppres. There were also significant increases in hip BA in all groups. At the femoral neck there was a 1.5 ±0.6% increase in BA in Nref ($p=0.03$). At the lumbar spine there was a -1.3 ±0.3% decrease in BA in Plow (<0.001). There were no significant changes in any measure of WBLH.

It is likely that total hip BA was affected by overlying fat and possibly by technical factors. The apparent increase in BA results in an increase in BMC and therefore may mask real changes in aBMD. The SA-BMC however corrects for changes in BA, and therefore provides a more representative analysis of changes in bone mineral at the total hip.

At the femoral neck and lumbar spine there are decreases in aBMD of >2% in Plow. At total hip, femoral neck and lumbar spine there were decreases in SA-BMC of 2.1% to 2.7% over 12 months in this group of young women. This exceeds the rates of bone loss seen in menopause, which are in the order of 1% per annum. The combination of rapid



increases in weight and significant decreases in bone mineral may increase the risk of lower limb fracture.

Changes in aBMD and SA-BMC are demonstrated in Plow from baseline to 12 months:

| **Plow** | | |
|---|---|---|
| Total hip | aBMD | +0.4 ±0.6% |
| | SA-BMC | -1.7 ±0.6% |
| Femoral neck | aBMD | -2.4 ±0.6% |
| | SA-BMC | -2.7 ±0.7% |
| Lumbar spine | aBMD | -2.0 ±0.6% |
| | SA-BMC | -2.1 ±0.6% |

There were no differences in vitamin D status between the groups over 12 months although Plow had less of a winter nadir than the other groups. Corrected calcium increased in all groups while albumin increased by 9.1 ±1.1% ($p<0.001$) in Plow, decreasing the differences seen between the groups at baseline. Serum ALP activity increased in all groups but increased by the largest amount (29.6 ±3.8%) in Plow. Serum phosphate increased by 7.0 ±2.3% in Nref but non-significantly in Ppres and Plow; therefore the original, baseline group differences remained at 12 months. TmP/GFR declined by 11.2 ±3.6% in Plow ($p=0.05$), and increased by 6.4 ±3.3% and 3.8 ±3.5% in Nref and Ppres respectively. However at 12 months Nref had a 9.9% greater TmP/GFR than Plow ($p=0.14$).

These analyses demonstrate that Plow is distinct from Nref and Ppres in measures of change in body composition, bone status, and markers of phosphate homeostasis. Plow had marked increases in weight and adiposity without commensurate increases in lean mass. The significant decreases in hip, femoral neck, and lumbar spine SA-BMC, and aBMD at the femoral neck and lumbar spine in Plow illustrate that Plow are losing mineral from these sites compared to the other groups. In contrast, Ppres and Nref are similar in most respects but distinct for serum phosphate and TmP/GFR. This is of interest because it might suggest that phosphate handling in Ppres is a precondition,

intermediate to Plow, and not yet reflected in other aspects of renal and bone homeostasis.

At baseline and at 12 months there were no significant differences in bone status between the groups when examined cross-sectionally. This analysis serves as a reminder of the importance of longitudinal follow-up of subjects when considering change in skeletal mineralisation, the differences demonstrated over time would be overlooked if only cross-sectional analyses were performed.

These observations suggest that Plow subjects are at a specific risk of metabolic and bone-related morbidity because of the rapid changes in body composition and bone mineral status.



# 8 *Post hoc* analysis on ART exposure

As indicated earlier (see Sections 4.11.3 and 4.11.4.7), given that there were changes in the composition of the three groups over the course of the study, the data presented in this chapter are the result of *post hoc* analyses. The subjects were analysed according to whether or not they were exposed to ART during the study period, rather than Ppres or Plow (and Nref). This approach is a way of clarifying that any changes seen in the Plow group were associated with ART exposure rather than other confounding factors.

Sections 8.1 to 8.2.2 present data consistent with the analysis in Chapter 6. Section 8.3 examines the ART-unexposed and –exposed in a longitudinal, hierarchical analysis similar to that in Chapter 7.

## 8.1 6-month visit

### 8.1.1 Anthropometry

At the 6-month visit the ART-unexposed group were significantly heavier (p=0.01) with greater median BMI (p=0.02) than ART-exposed. ART-unexposed had greater waist and hip circumferences (p≤0.02) and greater fat and lean mass (p≤0.05) compared with ART-exposed (Table 8-1).

**Table 8-1 Anthropometry at 6 months**

| | ART-unexposed *n*=66 | ART-exposed *n*=73 | Group effect ANOVA P = |
|---|---|---|---|
| Weight (kg) | 72.1 (17.3) | 65.0 (15.2) | 0.01 |
| BMI (kg/m$^2$) Median (IQR) | 27.0 (23.7;32.4) | 24.9 (21.6;29.4) | 0.02 |
| >24.9 kg/m$^2$ (%) | 67 | 47 | |
| >30 kg/m$^2$ (%) | 38 | 19 | |
| <18.5 kg/m$^2$ (%) | 2 | 8 | |
| Waist circumference (cm) | 89.6 (15.1)[1] | 82.4 (21.0) | 0.02 |
| Hip circumference (cm) | 108.9 (14.4) | 102.5 (12.2) | 0.006 |
| Waist:hip ratio | 0.81 (0.12) | 0.80 (0.16) | 0.75 |
| WBLH fat (kg) | 26.1 (10.5)[2] | 21.7 (8.9)[3] | 0.009 |
| WBLH lean (kg) | 39.2 (5.7)[2] | 37.5 (5.2)[3] | 0.05 |
| Fat:lean2 (kg/kg$^2$) | 16.4 (4.8)[2] | 15.0 (4.5)[3] | 0.08 |

All values are Mean (SD) unless indicated. cm, centimetres; IQR, interquartile range; kg, kilograms. [1]n=65, [2]n=64, [3]n=68

### 8.1.2 Bone measures

WBLH BMC and BA were significantly greater in ART-unexposed ($p \leq 0.005$), although after full size adjustment the differences in BMC were no longer apparent. There was no significant difference in aBMD (Table 8-2).

**Table 8-2 Bone mineral status at 6 months**

| | ART-unexposed *n*=64 | ART-exposed *n*=67 | Group effect ANOVA P = |
|---|---|---|---|
| **WBLH** | | | |
| BMC (g) | 1658 (249) | 1544 (228) | 0.007 |
| Area ($cm^2$) | 1724 (168) | 1647 (142) | 0.005 |
| BMD (g/ $cm^2$) | 0.958 (0.078) | 0.933 (0.078) | 0.07 |
| SA-BMC[a] (g) | 1593 (123) | 1588 (110) | 0.77 |

All values are Mean (SD). WBLH, whole body less head.
[a] Adjusted for weight & BA.

### 8.1.3 Vitamin D status

There were no significant group differences in vitamin D status at 6 months and the mean proportion with 25(OH)D greater or less than 50 nmol/l were comparable (Table 8-3).

**Table 8-3 Vitamin D status at 6 months**

| 25 (OH) D | ART-unexposed *n*=66 | ART-exposed *n*=74 | Group effect ANOVA P = |
|---|---|---|---|
| 25(OH)D (nmol/l) | 52.0 (18.6) | 54.4 (21.7) | 0.5 |
| 25(OH)D (nmol/l) >50, % | 67 | 70 | |
| 25(OH)D (nmol/l) <50, % | 33 | 30 | |
| 25(OH)D (nmol/l) <25, % | 5 | 3 | |

All values are Mean (SD) unless stated.
25(OH)D, 25 hydroxyvitamin D.

### 8.1.4 Biochemistry

There were significant differences in ALP activity at 6 months ($p \leq 0.001$) with ART-exposed having almost 25% higher concentrations. Similarly, there were differences ($P<0.0001$) in TmP/GFR with ART-unexposed being significantly higher than ART-exposed (Table 8-4).



**Table 8-4 Biochemistry at 6 months**

| Analyte/ derived variable | ART-unexposed *n*=66 | ART-exposed *n*=74 | Group effect ANOVA P = |
|---|---|---|---|
| Serum Cr (µmol/l) | 75.1 (13.1)[1] | 76.4 (13.7) | 0.08 |
| eGFR | 80.5 (19.8)[1] | 78.9 (21.9) | 0.24 |
| Serum albumin (g/l) | 39.8 (4.0)[2] | 38.6 (4.1) | 0.1 |
| Corrected calcium (mmol/l) | 2.38 (0.11)[2] | 2.36 (0.17) | 0.4 |
| Serum P (mmol/l) | 1.33 (0.25)[2] | 1.25 (0.28) | 0.08 |
| ALP (U/l) | 54.1 (22.1)[2] | 75.9 (28.6) | <0.001 |
| Urine P/Cr | 1.22 (0.7) | 1.15 (0.5)[4] | 0.47 |
| TmP/GFR | 1.50 (0.41)[3] | 1.39 (0.42)[4] | <0.0001 |

All values are Mean (SD).
ALP, alkaline phosphatase; Cr, creatinine; eGFR, estimated glomerular filtration rate; P, phosphate; TmP/GFR, renal tubular maximum reabsorption rate of phosphate to glomerular filtration rate.
[1]n=64, [2]n=65, [3]n=64, [4]n=73

## 8.2 12-month study visit

### 8.2.1 Anthropometry

By the 12-month visit, baseline differences in weight and BMI between the groups had diminished and there were no significant differences in mean anthropometric measures. Fat and lean mass differences had also become non-significant (Table 8-5).

**Table 8-5 Anthropometry at 12 months**

| | ART-unexposed *n*=62 | ART-exposed *n*=70 | Group effect ANOVA P = |
|---|---|---|---|
| Weight (kg) | 71.1 (16.7) | 66.6 (15.4) | 0.1 |
| BMI ($kg/m^2$) Median (IQR) | 26.3 (22.7; 31.4) | 25.5 (22.4;30.7) | 0.2 |
| BMI >24.9 $kg/m^2$ (%) | 66 | 49 | |
| BMI >30 $kg/m^2$ (%) | 35 | 20 | |
| BMI <18.5 $kg/m^2$ (%) | 2 | 7 | |
| Waist circumference (cm) | 90.3 (16.2)[1] | 88.3 (14.0)[3] | 0.4 |
| Hip circumference (cm) | 108.0 (13.6)[1] | 104.6 (11.9)[3] | 0.1 |
| Waist:hip ratio | 0.83 (0.07)[1] | 0.84 (0.07)[3] | 0.5 |
| WBLH fat (kg) | 25.6 (10.2)[2] | 23.9 (10.4)[4] | 0.3 |
| WBLH lean (kg) | 38.7 (5.6)[2] | 37.4 (5.5)[4] | 0.2 |
| Fat:lean2 ($kg/kg^2$) | 17.0 (5.6)[2] | 16.6 (4.9)[4] | 0.7 |

All values are Mean (SD) unless indicated. cm, centimetres; IQR, interquartile range; kg, kilograms.
[1]n=61, [2]n=59, [3]n=69, [4] n=68

### 8.2.2 Bone measures

Table 8-6 demonstrates bone variables at 12 months. ART-unexposed had significantly greater BMC at the LS and WBLH and aBMD at TH and LS. SA-BMC was also greater in this group at LS (Table 8-6).

**Table 8-6 Bone mineral status at 12 months**

| | ART-unexposed *n*=59 | ART-exposed *n*=68 | Group effect ANOVA P = |
|---|---|---|---|
| Total hip | | | |
| BMC (g) | 34.2 (5.9) | 33.2 (5.0) | 0.3 |
| Area ($cm^2$) | 33.4 (3.4) | 33.8 (3.2) | 0.4 |
| BMD ($g/cm^{2)}$ | 1.026 (0.139) | 0.981 (0.115) | 0.05 |
| SA-BMC[a] (g) | 33.9 (4.2) | 32.9 (3.3) | 0.11 |
| Femoral neck | | | |
| BMC (g) | 4.45 (0.67) | 4.30 (0.63) | 0.2 |
| Area ($cm^2$) | 4.78 (0.30 ) | 4.85 (0.34) | 0.2 |
| BMD ($g/cm^{2)}$ | 0.932 (0.136) | 0.887 (0.125) | 0.06 |
| SA-BMC[a] (g) | 4.46 (0.56) | 4.33 (0.52) | 0.18 |
| Lumbar spine | | | |
| BMC (g) | 58.2 (9.8) | 54.4 (9.1) | 0.02 |
| Area ($cm^2$) | 56.0 (4.8) | 55.2 (4.8) | 0.4 |
| BMD ($g/cm^{2)}$ | 1.036 (0.121) | 0.982 (0.122) | 0.01 |
| SA-BMC[a] (g) | 56.6 (6.0) | 54.2 (6.4) | 0.03 |
| WBLH | | | |
| BMC (g) | 1647 (238) | 1559 (221)[1] | 0.03 |
| Area ($cm^2$) | 1708 (146) | 1659 (144)[1] | 0.06 |
| BMD ($g/cm^{2)}$ | 0.962 (0.079) | 0.937 (0.073)[1] | 0.07 |
| SA-BMC[a] (g) | 1605 (119) | 1585 (110)[1] | 0.33 |

All values are Mean (SD). WBLH, whole body less head.
[a] Adjusted for weight & BA.
[1]n=67

### 8.2.3 Vitamin D status

At the 12-month visit there were no significant group differences in mean vitamin D status (Table 8-7).

**Table 8-7 Vitamin D status at 12 months**

| 25 (OH) D | ART-unexposed *n*=62 | ART-exposed *n*=68 | Group effect ANOVA P = |
|---|---|---|---|
| 25(OH)D (nmol/l) | 66.4 (17.4) | 61.1 (20.6) | 0.1 |
| 25(OH)D (nmol/l) >50, % | 66 | 71 | |
| 25(OH)D (nmol/l) <50, % | 34 | 29 | |
| 25(OH)D (nmol/l) <25, % | 5 | 3 | |

Values are Mean (SD) unless stated.
25(OH)D, 25 hydroxyvitamin D.



### 8.2.4 Biochemistry

There were significant differences in serum ALP activity at 12 months with ART-exposed having 30% higher concentrations. Serum albumin concentrations were not significantly different between the two groups. Serum phosphate was non-significantly higher in ART-unexposed. The resulting TmP/GFR was significantly reduced in the ART-exposed group (p=0.002), which was consistent with the pattern at six months (Table 8-8).

**Table 8-8 Biochemistry at 12 months**

| Analyte/ derived variable | ART-unexposed *n*=62 | ART-exposed *n*=69 | Group effect ANOVA P = |
|---|---|---|---|
| Serum Cr (µmol/l) | 74.4 (10.1) | 73.8 (10.4) | 0.7 |
| eGFR | 79.4 (13.2) | 80.2 (14.6) | 0.7 |
| Serum albumin (g/L) | 38.5 (4.0) | 39.6 (3.0) | 0.08 |
| Corrected calcium (mmol/L) | 2.34 (0.11) | 2.33 (0.16) | 0.6 |
| Serum P (mmol/L) | 1.31 (0.26) | 1.29 (0.28) | 0.6 |
| ALP (U/L) | 60.6 (21.5) | 93.4 (28.8) | <0.001 |
| Urine P/Cr | 1.21 (0.68) | 0.15 (0.11) | 0.02 |
| TmP/GFR | 1.47 (0.38) | 1.36 (0.45) | 0.002 |

All values are Mean (SD).
ALP, alkaline phosphatase; Cr, creatinine; eGFR, estimated glomerular filtration rate; P, phosphate; TmP/GFR, renal tubular maximum reabsorption rate of phosphate to glomerular filtration rate.

## 8.3 Longitudinal analysis

The remaining analyses in this chapter examine changes in body composition, bone and biochemical variables from baseline to 12 months in ART-unexposed and -exposed using the hierarchical, longitudinal models (Section 7.1). In the figures in this section ART-unexposed are represented in green, and ART-exposed in red.

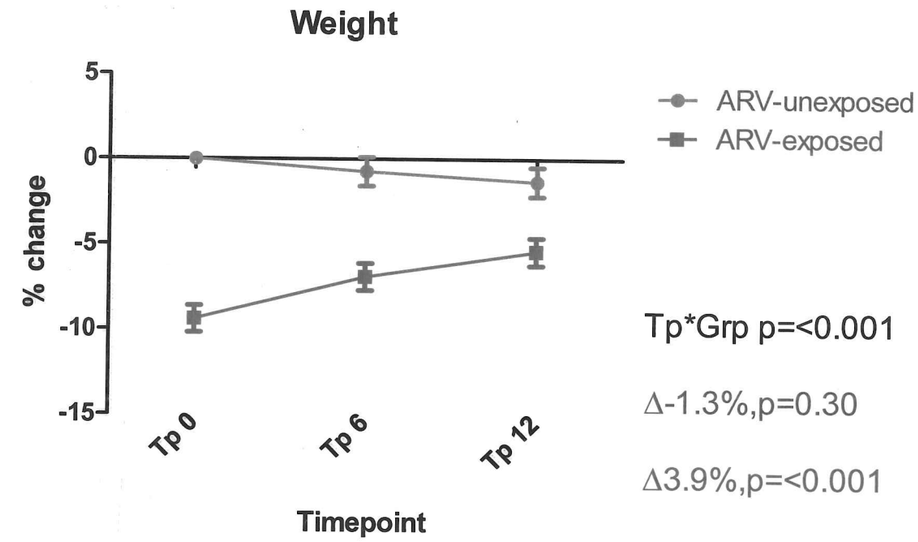
There was an increase in weight (3.9 ±0.81%) (p<0.001) and BMI (4.0 ±1.3%) (p=0.01) in ART-exposed, and non-significant decreases in ART-unexposed. Waist circumference was not significantly different from baseline, and while not significant, hip circumference had increased by 4.5 ±2.1% in ART-exposed. Corresponding WHR was -2.8 ±3.0% lower in this group compared to baseline (p=0.66) (Figure 8-1).

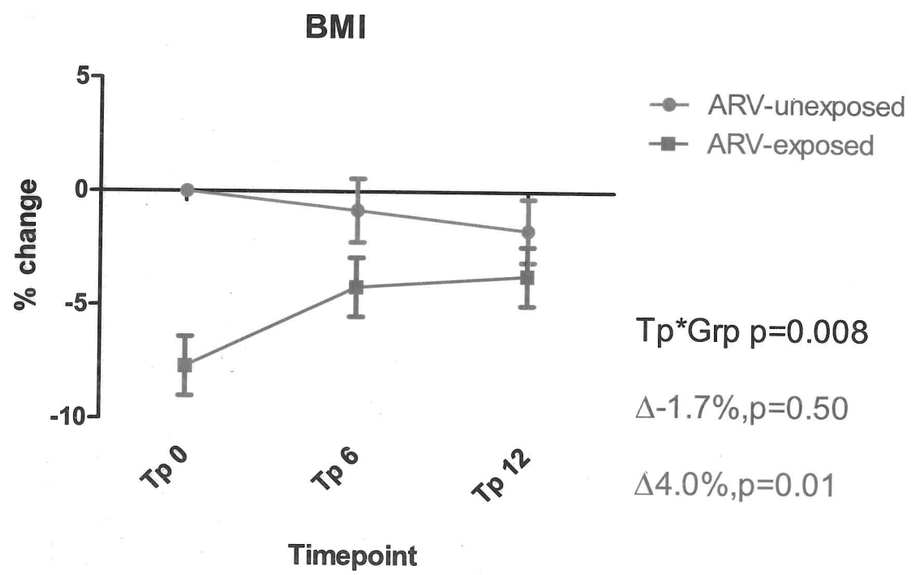
ART-exposed had mean increases in fat mass of 10.1 ±2.0% (p<0.001), which was highly significant and different from ART-unexposed, -2.1 ±2.1%, this was mirrored in fat:lean[2] ratios. ART-unexposed and ART-exposed had -1.9 ±0.6% (p=0.008) and 0.34 ±0.57 (p=0.8) changes in lean mass respectively (Figure 8-1).

These body composition changes very closely map, as anticipated, those differences seen in Ppres and Plow with ART-exposed being analogous to Plow.

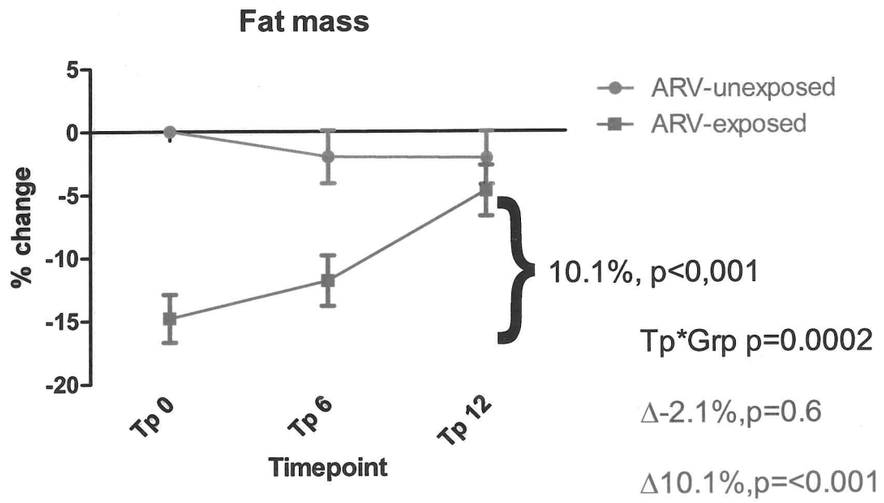


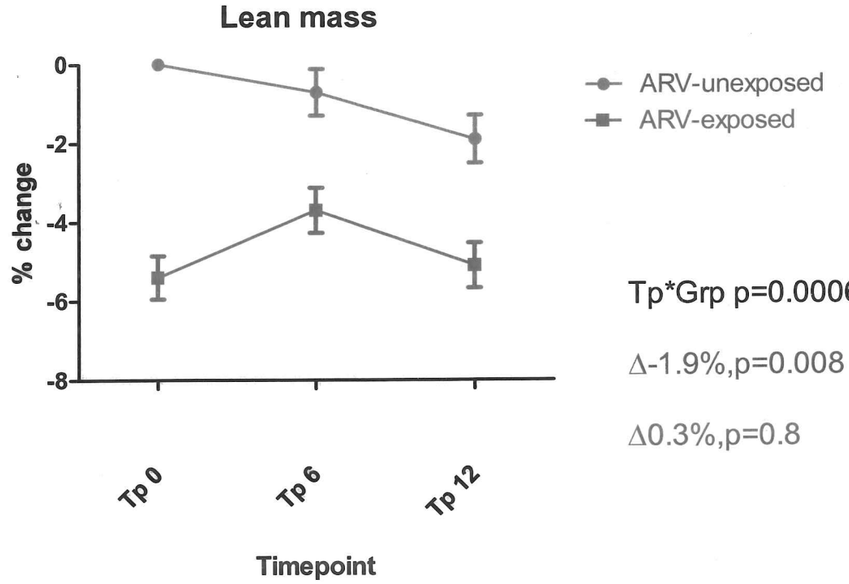
**Figure 8-1 Changes in body composition**



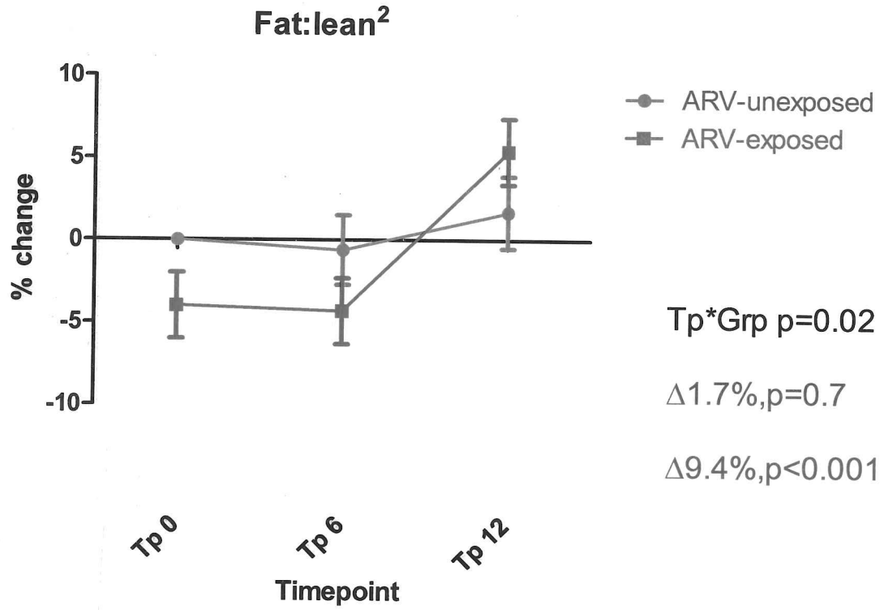

Percentage changes from baseline to 12 months in weight & BMI (ART-unexposed in green, ART-exposed in red). ART-unexposed at baseline is set at zero and ART-exposed is expressed relative to it.



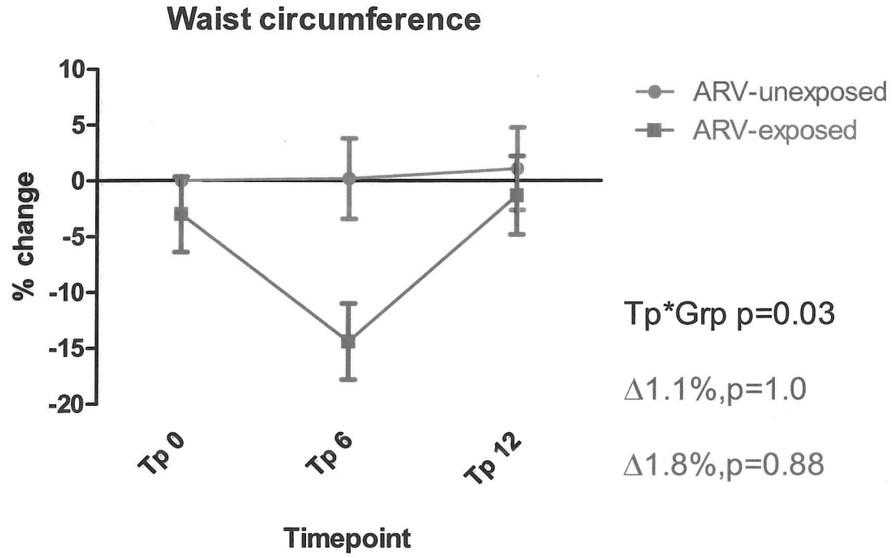



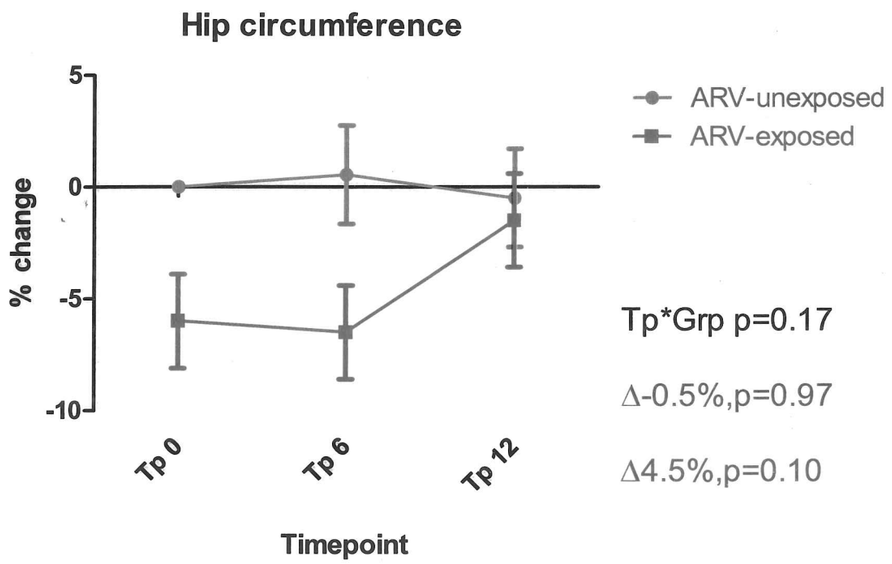

Percentage changes from baseline to 12 months in fat mass & lean mass (ART-unexposed in green, ART-exposed in red). ART-unexposed at baseline is set at zero and ART-exposed is expressed relative to it.





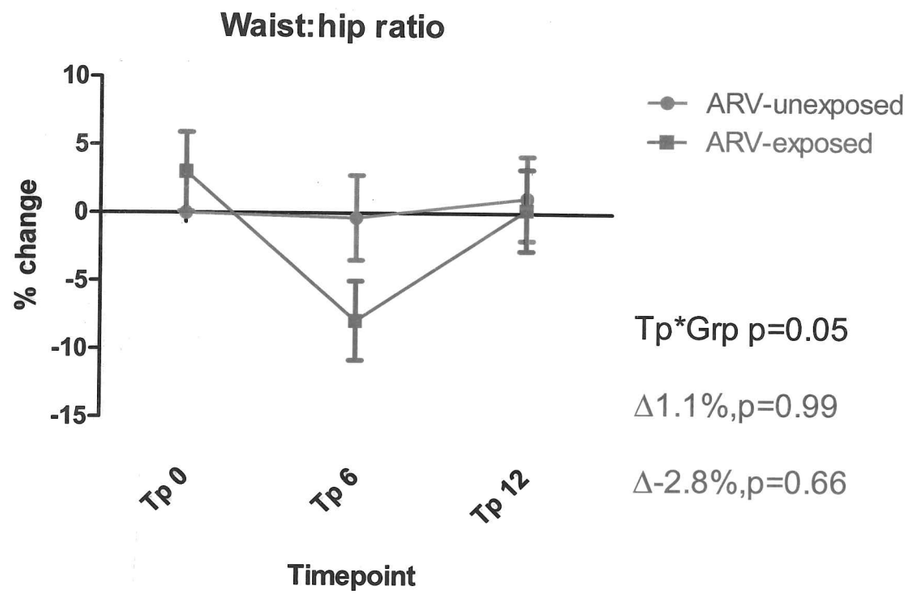

Percentage changes from baseline to 12 months in fat:lean² (ART-unexposed in green, ART-exposed in red). ART-unexposed at baseline is set at zero and ART-exposed is expressed relative to it.




Percentage changes from baseline to 12 months in waist & hip circumferences (ART-unexposed in green, ART-exposed in red). ART-unexposed at baseline is set at zero and ART-exposed is expressed relative to it.





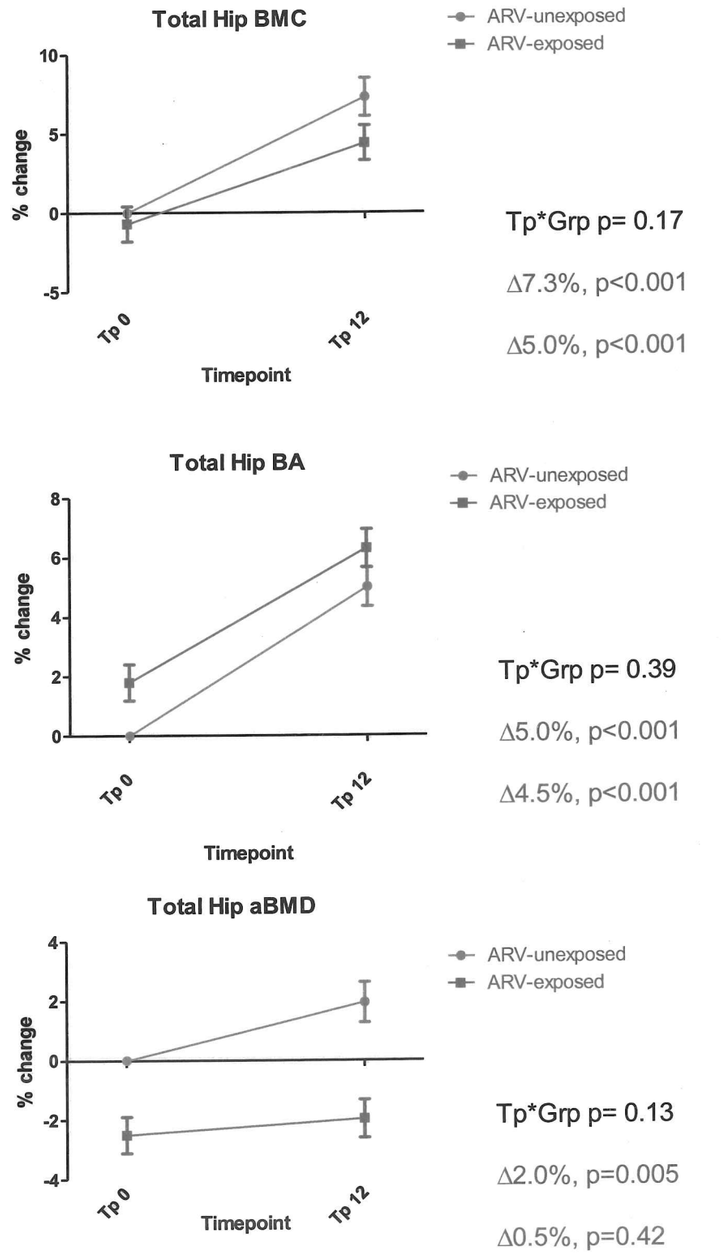

Percentage changes from baseline to 12 months in WHR (ART-unexposed in green, ART-exposed in red). ART-unexposed at baseline is set at zero and ART-exposed is expressed relative to it.

### 8.3.1 Bone measures longitudinal

#### 8.3.1.1 Total hip

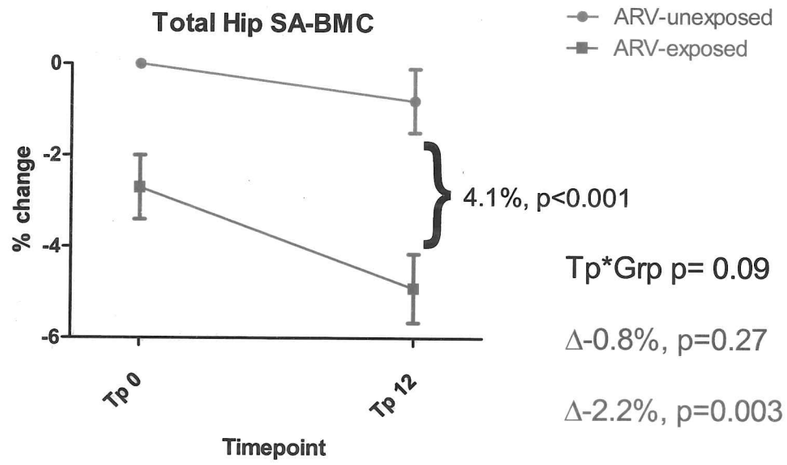
There were significant (p<0.001) increases in BMC and BA at the TH in both groups. In ART-unexposed there was a 2.0 ±0.68% (p=0.005) increase in aBMD with a non-significant increase in ART-unexposed. There were decreases at the total hip in SA-BMC in ART-unexposed -0.80 ±0.73% (NS) and ART-exposed -2.2 (0.74)% (p=0.003) with a non-significant interaction term (p=0.09). At 12 months SA-BMC in ART-exposed was 4.1 (0.75)% (p<0.001) greater than ART-unexposed (Figure 8-2).

Figure 8-2 Changes in hip




Percentage changes from baseline to 12 months in total hip bone measures (BMC, BA & aBMD) (ART-unexposed in green, ART-exposed in red). ART-unexposed at baseline is set at zero and ART-exposed is expressed relative to it.



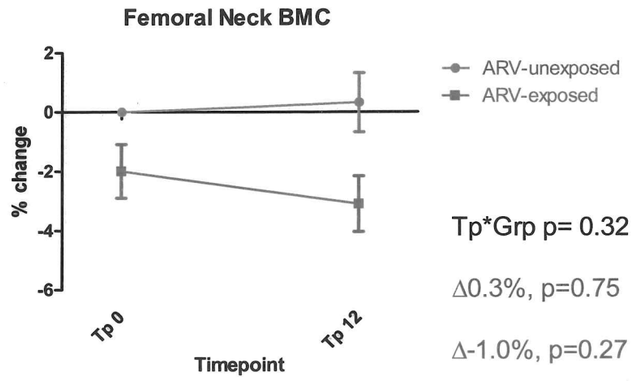


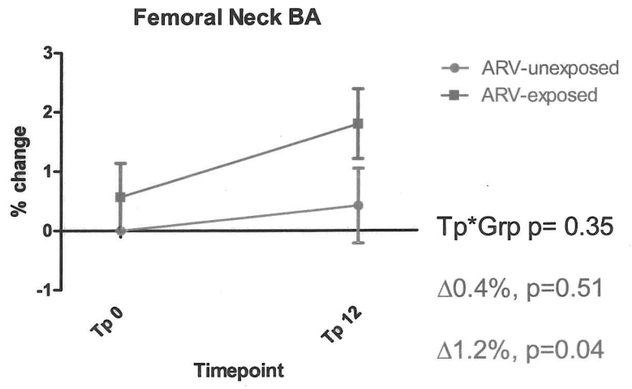

Percentage changes from baseline to 12 months in total hip bone measures (SA-BMC) (ART-unexposed in green, ART-exposed in red). ART-unexposed at baseline is set at zero and ART-exposed is expressed relative to it.

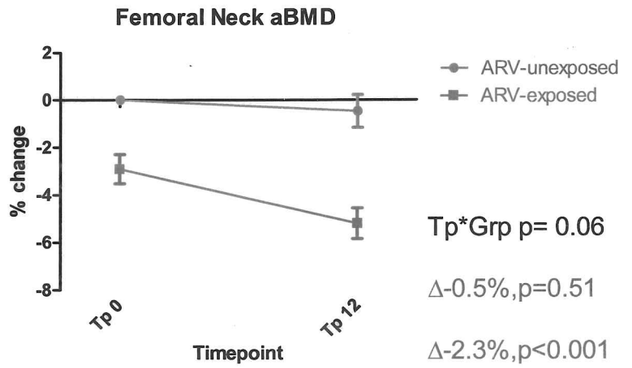
### 8.3.1.2 Femoral neck

At the FN there was a non-significant increase in BMC in ART-unexposed 0.33 ±1.0, and a non-significant decrease in ART-exposed -1.0 ±0.94%. There were increases in BA in both groups; 0.42 ±0.63% (NS) and 1.2 ±0.59% (p=0.04) in ART-unexposed and ART-exposed respectively. There were decreases in aBMD in both groups with a significant decrease in ART-exposed -2.3 ±0.65% (p<0.001). There was a decrease in SA-BMC in ART-unexposed -0.32 ±0.70% (NS) and ART-exposed, -2.7 ±0.70% (p<0.001). At 12 months there was a 4.8% (p<0.001) difference between ART-exposed and ART–unexposed in SA-BMC (Figure 8-3).

Figure 8-3 Changes in femoral neck

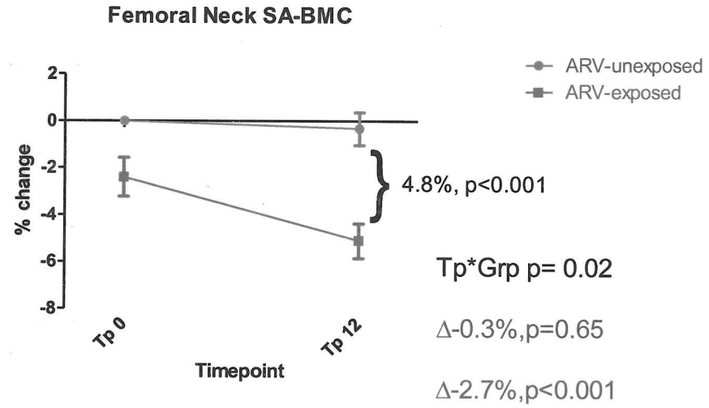



Percentage changes from baseline to 12 months in femoral neck bone measures (BMC, BA & aBMD) (ART-unexposed in green, ART-exposed in red). ART-unexposed at baseline is set at zero and ART-exposed is expressed relative to it.



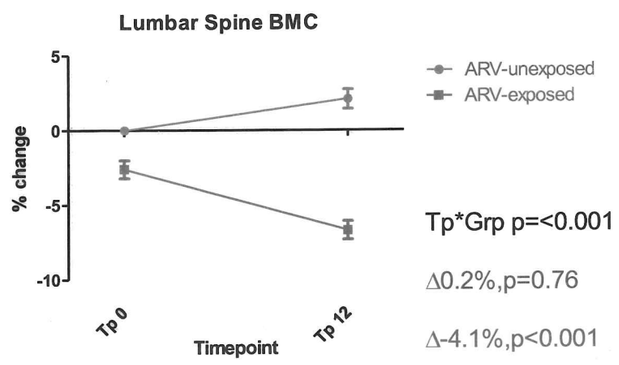


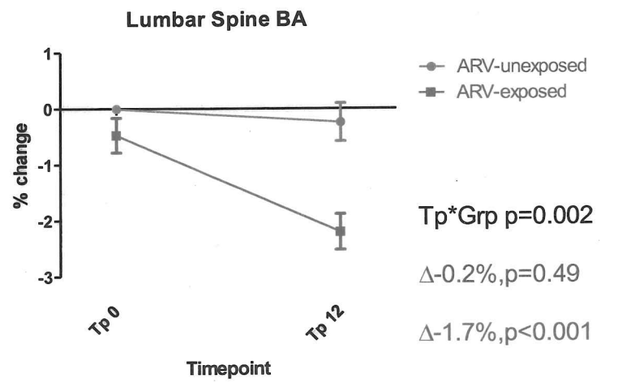

Percentage changes from baseline to 12 months in femoral neck bone measures (SA-BMC) (ART-unexposed in green, ART-exposed in red). ART-unexposed at baseline is set at zero and ART-exposed is expressed relative to it.

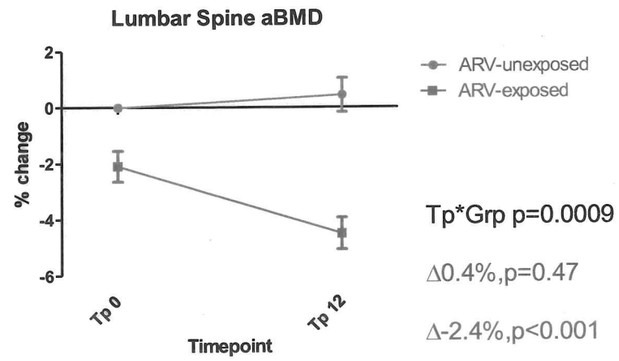
#### 8.3.1.3 Lumbar spine

There were non-significant changes in BMC, BA, aBMD, and SA-BMC in ART-unexposed groups at the LS. The ART-exposed group had a -4.1 ±0.62% (p<0.001) decrease in BMC and a -1.7 ±0.32% (p<0.001) decrease in BA. There was a significant decrease in aBMD in ART-exposed -2.4 ±0.57 (<0.001). There was a decrease in SA-BMC in ART-exposed -2.8 ±0.65% (p<0.001). At 12 months there was a 5.2% (p<0.001) difference between ART-unexposed and ART–exposed in SA-BMC (Figure 8-4).

**Figure 8-4 Changes in lumbar spine**

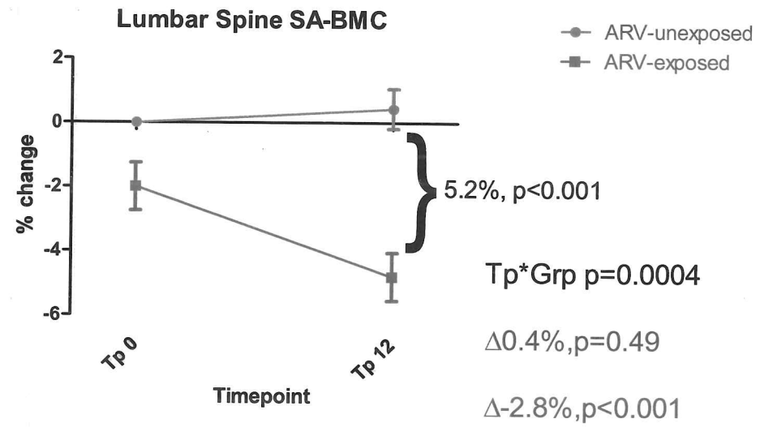



Percentage changes from baseline to 12 months in lumbar spine bone measures (BMC, BA & aBMD) (ART-unexposed in green, ART-exposed in red). ART-unexposed at baseline is set at zero and ART-exposed is expressed relative to it.



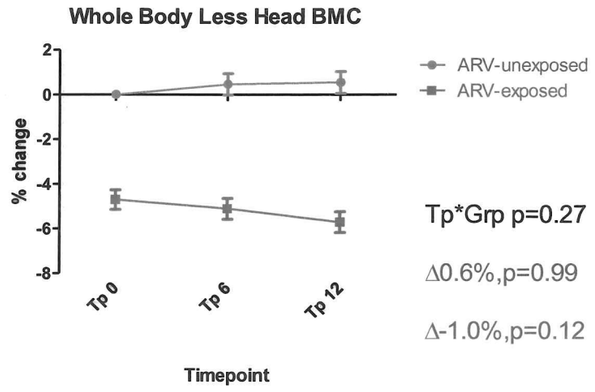


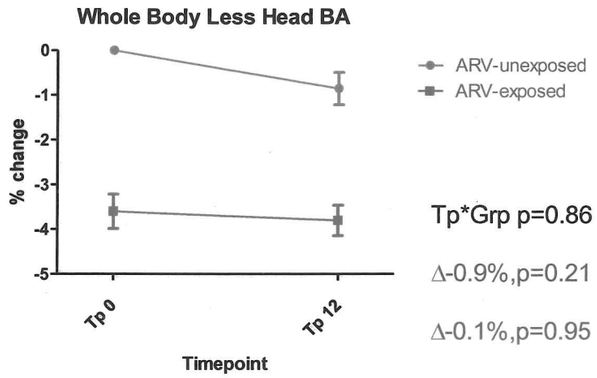

Percentage changes from baseline to 12 months in lumbar spine bone measures (SA-BMC) (ART-unexposed in green, ART-exposed in red). ART-unexposed at baseline is set at zero and ART-exposed is expressed relative to it.

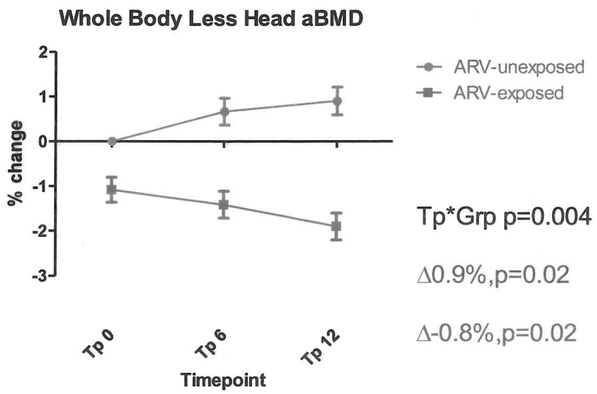
#### 8.3.1.4 Whole body less head

There were non-significant changes in BMC and BA in both groups at WBLH. There was a significant increase in aBMD in ART-unexposed 0.90 ±0.31% (p=0.02) and a significant decrease in ART-exposed -0.83 ±0.30 (p=0.02). ART-unexposed had a 0.89 ±0.32% increase (p=0.02) in SA-BMC and a significant interaction (p=0.05), there was a non-significant decrease in ART-exposed. At 12 months there was a 2.8% (p<0.001) difference between ART-unexposed and ART–exposed in SA-BMC (Figure 8-5).

**Figure 8-5 Changes in whole body**

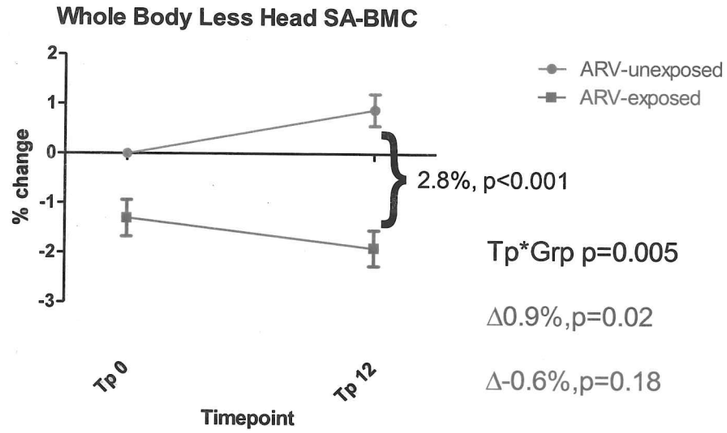



Percentage changes from baseline to 12 months in whole body less head bone measures (BMC, BA & aBMD) (ART-unexposed in green, ART-exposed in red). ART-unexposed at baseline is set at zero and ART-exposed is expressed relative to it.



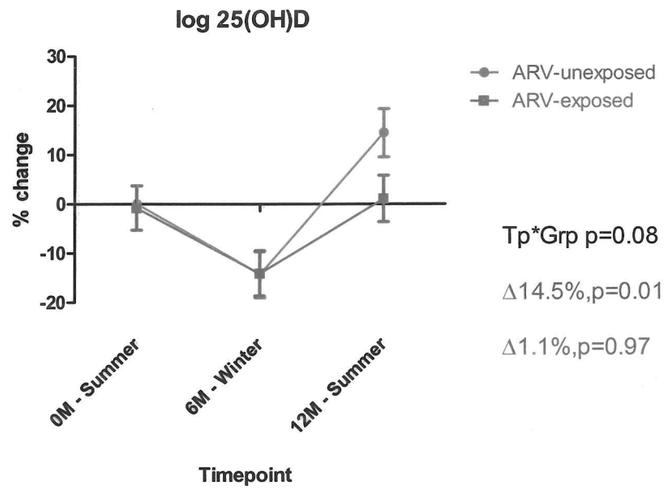



Percentage changes from baseline to 12 months in whole body less head bone measures (SA-BMC) (ART-unexposed in green, ART-exposed in red). ART-unexposed at baseline is set at zero and ART-exposed is expressed relative to it.

### 8.3.2 Vitamin D status

In ART-unexposed and ART–exposed there were mean winter decreases of 25(OH)D of 14.2 ±4.8% and 14.1 ±4.5% respectively. By 12 months (summer) the ART-unexposed had increased significantly from baseline by 14.5 ±4.9% (p=0.01) but with a non-significant interaction (p=0.08). There was no significant change in 25(OH)D in ART-exposed over 12 months (Figure 8-6).

**Figure 8-6 Changes in vitamin D status**




Percentage changes from baseline to 12 months in vitamin D status (ART-unexposed in green, ART-exposed in red). ART-unexposed at baseline is set at zero and ART-exposed is expressed relative to it.

#### 8.3.2.1 Vitamin D and fat mass

To explore percentage changes in 25(OH)D and any relationship to adiposity the following model was constructed for ART-exposed:

Y variable: log 25(OH)D at 12 months – log 25(OH)D at baseline

X variables:

- group
- log 25(OH) D at baseline (to prevent regression to the mean)
- log fat mass at 12 months – log fat mass at baseline (lnfat12-lnfat0)
- mean (log fat mass at 12 months + log fat mass at baseline)

Also, fat mass did not predict 25(OH)D concentration at six or 12 months. Change in fat did not predict change in vitamin D status. There were non-significant coefficients and interaction terms ($p>0.05$) at both time points. The group*lnfat12-lnfat0 interaction was non-significant, $p=0.5$.

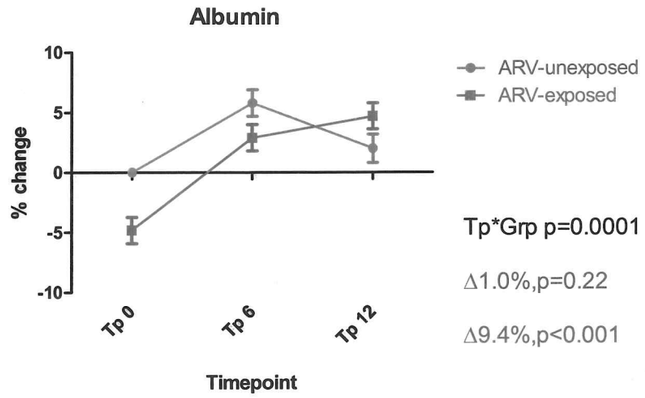


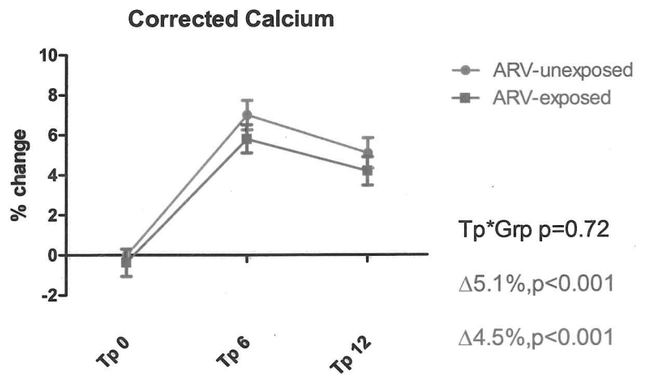
### 8.3.3 Biochemistry

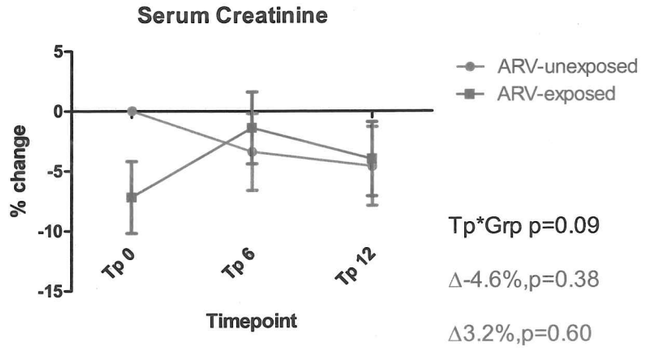
There were increases in serum albumin concentrations from baseline in ART-exposed, 9.4 ±1.1% ($p<0.001$), with non-significant increases in ART-unexposed over 12 months. There were significant increases in corrected calcium in both groups but non-significant interaction terms ($p>0.05$). Serum creatinine concentrations and eGFR did not change significantly in either group.

There were large, significant increases in ALP activity, with the largest increases seen in ART-exposed, 34.8 ±3.7% ($p<0.001$) (Figure 8-7).

**Figure 8-7 Changes in biochemistry and derived variables**

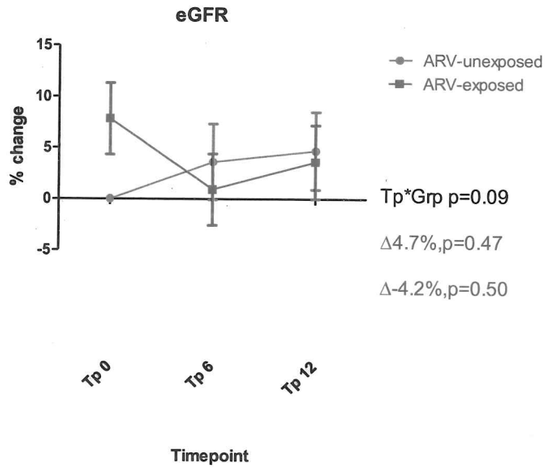


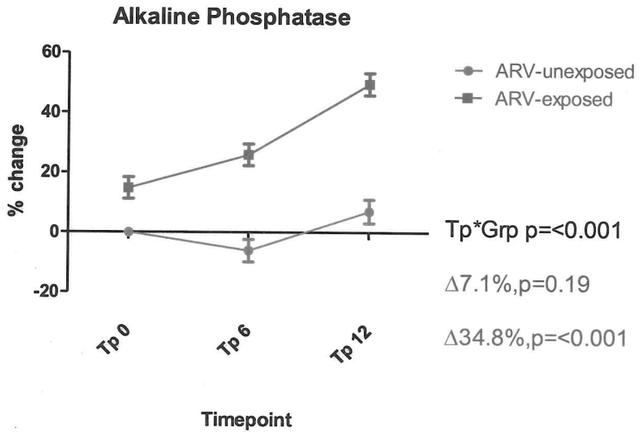




Percentage changes from baseline to 12 months in biochemical analytes and derived variables (ART-unexposed in green, ART-exposed in red). ART-unexposed at baseline is set at zero and ART-exposed is expressed relative to it.






Percentage changes from baseline to 12 months in biochemical analytes and derived variables (ART-unexposed in green, ART-exposed in red). ART-unexposed at baseline is set at zero and ART-exposed is expressed relative to it.

### 8.3.4 Changes in markers of phosphate homeostasis

When analysed at baseline, ART-exposed had significantly higher mean serum phosphate concentrations than ART-unexposed ($p \leq 0.05$). At 12 months there was an overall increase in serum phosphate in ART-unexposed, 7.9 ±2.7% ($p=0.003$), but no significant change in ART-exposed -1.9 ±2.5% (Figure 8-8). At baseline, ART-unexposed had a significantly lower TmP/GFR when compared to ART-exposed 1.37 ±0.35%, 1.58 ±0.49% respectively, ($p=0.003$). At 12 months ART-unexposed had a 9.8 ±3.9%

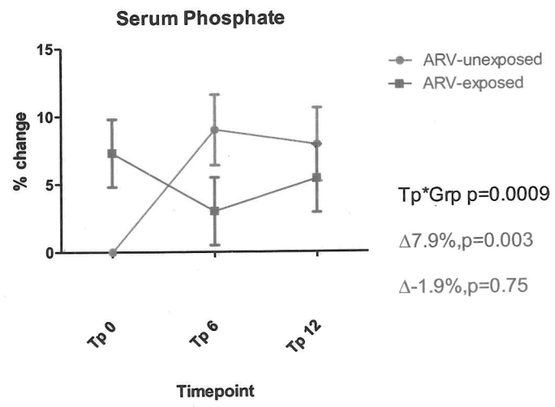
increase in TmP/GFR (p=0.04) compared to a 15.4 ±3.7% decrease in ART-exposed (p=0.0002). These findings are consistent with the observed changes in serum phosphate concentrations (Table 8-9).

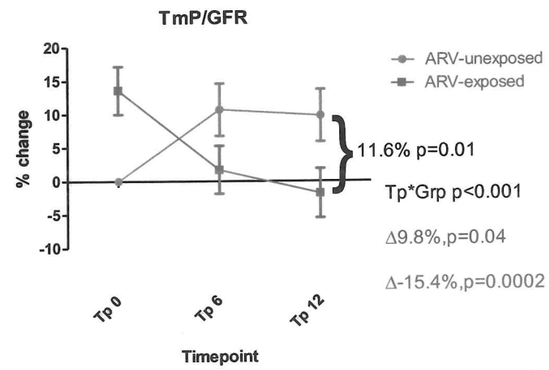
**Table 8-9 Changes in phosphate**

| | | |
|---|---|---|
| **ART-unexposed** | Serum phosphate | ↑ |
| | TmP/GFR | ↑ |
| **ART-exposed** | Serum phosphate | ↓/↔ |
| | TmP/GFR | ↓ |

Relative changes in markers of phosphate metabolism in ART-unexposed and ART-exposed

**Figure 8-8 Changes in phosphate metabolism**




Percentage changes from baseline to 12 months in markers of phosphate homeostasis (ART-unexposed in green, ART-exposed in red). ART-unexposed at baseline is set at zero and ART-exposed is expressed relative to it.



## 8.4 Summary

In summary, the changes seen in body composition and bone variables were similar in the groups categorised *post hoc* as ART-unexposed and ART-exposed, when compared to the original allocation Ppres and Plow (see Section 7.1). There were significant increases in weight, BMI, fat mass, and fat:lean$^2$ in ART-exposed. The ART-unexposed had a significant decrease in lean mass (Table 8-10).

**Table 8-10 Changes in anthropometry and body composition**

| Variable | ART-unexposed | ART-exposed |
|---|---|---|
| Weight (kg) | -1.3 (0.9) | +3.9 (0.8) **<0.001** |
| BMI (kg/m$^2$) | -1.7 (1.4) | +4.0 (1.3) **0.01** |
| Fat (kg) | -2.1 (2.1) | +10.1(2.0) **<0.001** |
| Lean (kg) | -1.9 (0.6) **0.008** | +0.3 (0.6) |
| Fat:lean$^2$ | +1.7 (2.2) | +9.4 (2.0) **<0.001** |

Values are mean (SE) percentage change from baseline to 12 months in each group, *p*= in bold type. Where no *p* value stated, *p*>0.05.

There were significant increases in hip BMC and BA in both groups; ART-unexposed had a significant increase in aBMD. However, when full size-adjustment was conducted ART-exposed had a significant decrease in SA-BMC. At the femoral neck the ART-exposed group had increases in BA and decreases in aBMD and SA-BMC. At the lumbar spine there were significant decreases in all bone variables in ART-exposed. At WBLH there were increases in aBMD and SA-BMC in ART-unexposed, and a decrease in aBMD in ART-exposed (Table 8-11).

Table 8-11 represents the same data as Table 7-2 but for ART-unexposed and ART–exposed groups.

Table 8-11 Changes in bone mineral status

| Variable | ART-unexposed | ART-exposed |
|---|---|---|
| **Total hip** | | |
| BMC (g) | +7.3 (1.2) **<0.001** | +5.0 (1.1) **<0.001** |
| BA ($cm^2$) | +5.0 (0.7) **<0.001** | +4.5 (0.6) **<0.001** |
| BMD ($g/cm^2$) | +1.95 (0.7) **0.005** | +0.5 (0.6) |
| SA-BMC (g) | -0.80 (07) | -2.2 (0.7) **0.003** |
| **Femoral neck** | | |
| BMC (g) | +0.33 (1.0) | -1.0 (0.9) |
| BA ($cm^2$) | +0.42 (0.6) | +1.2 (0.6) **0.04** |
| BMD ($g/cm^2$) | -0.46 (0.7) | -2.3 (0.7) **<0.001** |
| SA-BMC (g) | -0.32 (0.7) | -2.7 (0.7) **<0.001** |
| **Lumbar spine** | | |
| BMC (g) | +0.21 (0.7) | -4.1 (0.6) **<0.001** |
| BA ($cm^2$) | -0.24 (0.4) | -1.7 (0.3) **<0.001** |
| BMD ($g/cm^2$) | +0.44 (0.6) | -2.4 (0.6) **<0.001** |
| SA-BMC (g) | +0.43 (0.6) | -2.8 (0.7) **<0.001** |
| **WBLH** | | |
| BMC (g) | +0.55 (0.5) | -0.96 NS |
| BA ($cm^2$) | -0.85 (0.4) | -0.13 (0.3) |
| BMD ($g/cm^2$) | +0.90 (0.3) **0.02** | -0.83 (0.3) **0.02** |
| SA-BMC (g) | +0.89 (0.3) **0.02** | -0.58 (0.3) |

Values are mean (SE) percentage change from baseline to 12 months in each group, *p*= in bold type. Where no *p* value stated, *p*>0.05.

In the ART-unexposed group there was a significant increase in 25(OH)D and serum phosphate over the course of the study period but no significant change in the ART-exposed group. There were increases in corrected calcium and no significant change in serum creatinine and eGFR. In ART-exposed there were large increases in ALP activity and urine phosphate to creatinine ratio. TmP/GFR significantly increased by 9.8 ±3.9% in ART-unexposed and decreased by 15.4 ±3.7% in ART-exposed (p=0.0002) (Table 8-12). Table 8-12 represents the same data as Table 7-3 but for ART-unexposed and ART–exposed groups.



Table 8-12 Changes in vitamin D status and biochemistry

| Variable/ derived variable | ART-unexposed | ART-exposed |
|---|---|---|
| 25(OH)D (mmol/l) | +14.5 (4.9) **0.01** | +1.1 (4.7) |
| Corrected calcium (mmol/l) | +5.1 (0.75) **<0.001** | +4.5 (0.71) **<0.001** |
| Albumin (g/l) | +1.0 (1.2) | +9.4 (1.1) **<0.001** |
| ALP (U/l) | +7.1 (3.9) | +34.8 (3.7) **<0.001** |
| Serum P (mmol/l) | +7.9 (2.7) **0.003** | -1.9 (2.5) |
| Urine P/Cr | -1.4 (13.1) | +58.2 (12.3) **<0.001** |
| Serum Cr (µmol/l) | -4.6 (3.3) | +3.2 (3.1) |
| eGFR | +4.7 (3.8) | -4.2 (3.6) |
| TmP/GFR | +9.8 (3.9) **0.04** | -15.4 (3.7) **0.002** |

Values are mean (SE) percentage change from baseline to 12 months in each group, *p*= in bold type. Where no *p* value stated, *p*>0.05.
ALP, alkaline phosphatase; Cr, creatinine; eGFR, estimated glomerular filtration rate; P, phosphate; TmP/GFR, renal tubular maximum reabsorption rate of phosphate to glomerular filtration rate.

These *post hoc* analyses demonstrate a similar magnitude of change in terms of body composition when compared with the three group analysis comparing Ppres and Plow (and Nref). They also reveal a more pronounced change in bone status over time with greater declines in aBMD at the lumbar spine and greater declines in SA-BMC at the total hip and lumbar spine.

This type of analysis also highlighted relative differences in vitamin D status at 12 months with ART-unexposed seeing a 14.1% (p=0.01) increase in 25(OH)D compared to a non-significant increase of 1.1% in ART-exposed. There were also larger increases in serum albumin and ALP activity in ART-exposed compared to ART–unexposed. Significant differences in TmP/GFR are revealed in this *post hoc* analysis, so that ART-exposed saw a 15.4% decrease while ART–unexposed a 9.8% increase in this measure of renal phosphate handling. This result was accompanied by a non-significant decrease in serum phosphate in ART-exposed which is in keeping with the hypothesis of ART-induced renal phosphate wasting.

Therefore this *post hoc* analysis provides interesting insights into the differences in those exposed and unexposed to ART beyond those demonstrated by original grouping.

# 9 Discussion

The study that forms the basis of this thesis was carried out in Soweto, an impoverished, urban area of Johannesburg, the largest city in South Africa. Soweto has a very high prevalence of HIV infection, ranging from 19.4% in the general population (Piwowar-Manning *et al*, 2011) to >35% in pregnant women (Gounder *et al*, 2011). South Africa is described as being a hyper-endemic region for HIV infection with high rates of new infections. Historically, Soweto has seen high rates of under-nutrition and stunting, and malnutrition remains prevalent (Willey *et al*, 2009). Relatively recently there have been dramatic secular changes in Soweto, and urban South Africa more generally, with increases in childhood and adult obesity, and burgeoning non-communicable disease burden. These two epidemics overlap so that HIV infection may be partially associated with non-communicable disease or may be a direct cause, for example HIV-associated cardiomyopathy (Sliwa *et al*, 2012).

In recent years, access to free ART treatment has dramatically improved life expectancy in South Africa with estimates of up to 11.3 years gained with the introduction of ART, in as little as 11 years since the roll-out of ART in 2000 (Bor *et al*, 2013). For this reason it can be predicted that South Africa will continue to see rises in life expectancy in HIV-positive populations, so much so that they are likely to survive for long enough to develop non-communicable disease, such as osteoporosis, seen with advancing age in other populations. If, as has been demonstrated in Caucasian populations, ART exposure is associated with poor bone health it is important to describe the history of this (in a South African population) in order to anticipate the magnitude of the problem and potential consequences for population and individual health within Africa (Bendavid *et al*, 2012).

The work described in this thesis has sought to gain insights into the associations between HIV infection, ART exposure, body composition, bone mineral, and vitamin D status in women in Soweto. It also sought to explore changes in body composition, bone



mineral, and vitamin D status over time. The study was designed to test several key hypotheses that were developed after scrutinising the existing literature on body composition, bone health, and vitamin D status in HIV-positive individuals. The data from these wide-ranging studies are mixed, particularly with respect to changes in bone density.

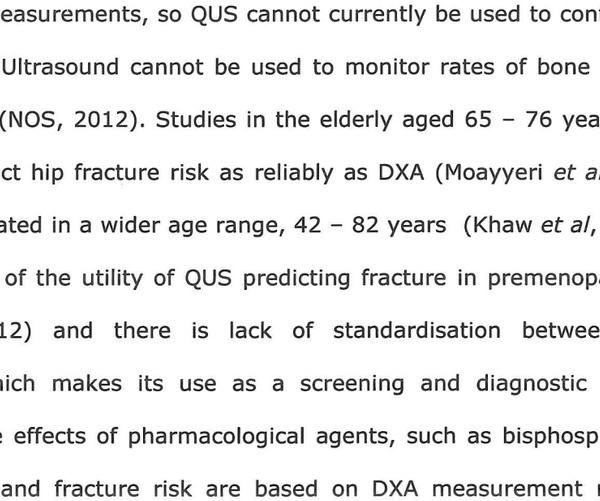
The majority of published studies demonstrated low bone mass associated with HIV infection and ART exposure (Brown *et al*, 2006b), although some did not support these observations and indeed saw the opposite effect. Work by Bolland *et al* described either no bone loss associated with ART exposure or an increase, at least initially, of aBMD after initiation of ART (Bolland *et al*, 2006; 2011). Data generated from this study support the idea that tenofovir-exposure is most likely driving loss of bone mineral in these women. It supports other data such as that described in the PrEP study (Liu *et al*, 2011) demonstrating loss of bone mineral following tenofovir-exposure in HIV negative men, and the 'ASSERT' study (Stellbrink *et al*, 2010) which described higher rates of bone loss with tenofovir- than abacavir-based ART in HIV-infected patients. Until recently there has been little attention paid to bone health in HIV-positive Africans living in Africa. At the time of writing this thesis there was only one paper examining the effect of HIV infection on bone density in Africa; this was a 2012 publication from Senegal describing a cross-sectional study examining the association of ART-use (mean duration 8.8 years, 45% were receiving a protease inhibitor-based regime) using QUS at the calcaneus. The HIV-positive patients in this study had lower bone density by QUS than controls (Figure 9-1).

QUS has been used either alone or in conjunction with DXA to assess fracture risk (Chan *et al*, 2012; 2013). QUS gives a measure of microstructure of bone (particularly at the calcaneus) which closely correlates with BMD. It has the advantages of being relatively inexpensive and portable when compared with DXA and does not involve the use of ionising radiation. However, osteoporosis is defined by the WHO in terms of bone density measured by DXA. "There is currently no established, accepted definition of osteoporosis

based on QUS measurements, so QUS cannot currently be used to confirm the diagnosis of osteoporosis. Ultrasound cannot be used to monitor rates of bone loss or bone gain with treatment" (NOS, 2012). Studies in the elderly aged 65 – 76 years suggested that QUS might predict hip fracture risk as reliably as DXA (Moayyeri *et al*, 2009), and this finding was repeated in a wider age range, 42 – 82 years (Khaw *et al*, 2004). There is a lack of evidence of the utility of QUS predicting fracture in premenopausal women and men (NOS, 2012) and there is lack of standardisation between different QUS technologies, which makes its use as a screening and diagnostic tool problematic. Furthermore, the effects of pharmacological agents, such as bisphosphonates, on bone mineral density and fracture risk are based on DXA measurement rather than QUS, therefore QUS has not been validated to demonstrate effects of treatment on BMD and fracture risk.

In the paper from Senegal cited, Cournil *et al* described a heterogeneous group of patients with a mean age of 47.0 years and a variety of different ART exposures; whilst an important study in assessing bone health in African subjects it is limited by design and choice of imaging modality.

**Figure 9-1 Differences in BMD in Senegalese subjects**




"Mean QUS bone mineral density expressed as T-score (and 95% confidence interval) in HIV-infected patients and controls, separately for men and women" (Cournil *et al*, 2012). [Content under open content licenses may be reused without any need to contact the licensor. See: http://creativecommons.org/licenses/by/2.5/]



The dearth of African studies examining the association of HIV infection and ART exposure with low bone mineral status aside, it seems reasonable to speculate that HIV-positive African women experience low bone mineral or bone mineral loss. The results of the study presented in this thesis certainly support an association between low $CD_4$ count, ART exposure, and bone mineral loss. There were rates of decline in aBMD and SA-BMC of ≥2% at the lumbar spine over 12 months in Plow (as well as in ART-exposed) when compared with baseline. Indeed, at baseline there were no significant differences in bone status between any of the three groups (Nref, Ppres, and Plow) (see Section 9.2).

In contrast to bone health, there are more published studies examining the effect of HIV infection and ART exposure on body composition in African subjects. A number of studies in children and adults with older, stavudine-containing ART combinations have associated ART use with accumulation of visceral fat and loss of limb fat (Innes *et al*, 2012; Goedecke *et al*, 2013). Studies have also demonstrated comparatively lower lean mass in some HIV-positive Africans than expected (Mutimura *et al*, 2010; Mupere *et al*, 2012). Some of these studies have used DXA to examine body composition but are cross-sectional and do not establish baseline body composition characteristics and change after ART-initiation. Awareness of the importance of examining body composition in HIV-positive African subjects and those exposed to ART is in part due to the appreciation of an increase in metabolic dysregulation associated with HIV infection (Boufassa *et al*, 2012) and the likely burden of associated cardio-metabolic disease.

The studies examining vitamin D status in HIV-positive patients almost universally describe high rates of vitamin D deficiency (Van Den Bout-Van Den Beukel *et al*, 2008a; Childs *et al*, 2012). There have been many studies into the immune-modulating effects of vitamin D over recent years and a clinical perception that low vitamin D status is associated with increased risk for infectious disease and an impaired ability to combat these infections should they be acquired. For this reason, as well as those pertinent to

bone health, it was important to investigate vitamin D status in an urban, South African context.

One of the key findings of the research presented in this thesis is that, at baseline, HIV-positive women did not differ from HIV-negative women in measures of bone and vitamin D status, and only in Plow in weight and measures of fat. The same findings were also described at six and 12 months when examined cross-sectionally. However, the more detailed longitudinal analysis demonstrated within individual declines in aBMD and SA-BMC at the femoral neck and lumbar spine of ≥2.0%, and SA-BMC at the hip of 1.7% in Plow suggesting a rate of bone loss similar to, or greater than, at menopause. Similar results were obtained when the Ppres and Plow groups were reclassified as ART-unexposed and ART-exposed. In ART-exposed there were complementary decreases in aBMD and SA-BMC at the femoral neck compared to Plow with decreases of -2.3% compared with -2.4% in aBMD and -2.7% in Plow and ART-exposed in SA-BMC. At the lumbar spine there was a greater decrease in aBMD and SA-BMC in ART-exposed compared to Plow by -0.4% and -0.7% respectively. At the hip there was a decline in SA-BMC of 2.2% in ART-exposed compared to 1.7% in Plow.

The *post hoc* analysis examining ART-unexposed and ART–exposed proved slightly more discriminating than examining differences over time using the baseline Ppres and Plow classification. The reason analysing subjects by ART exposure status allowed a more precise discernment of the effects of ART, or of declining health for those in Ppres who had falls in $CD_4$ count, on body composition, bone, and vitamin D status than by original classification was cross-over between groups that occurred over the course of the study. This was due to several factors including: i) increases and decreases in individual $CD_4$ counts, which changed their eligibility for ART, ii) individuals due to start ART after the baseline visit not commencing therapy as planned, and iii) a change in South African $CD_4$ eligibility criterion for commencing ART from ≤ 200 $x10^6$ cells/l to ≤350 $x10^6$ cells/l.



This thesis adds to the body of knowledge on the subject of HIV infection, immune dysregulation, ART exposure, body composition, bone health, and vitamin D status in the following areas.

## 9.1 Body composition

At baseline, Plow was significantly lighter, with a lower BMI, than Nref and Ppres, although rates of overweight and obesity were generally high in all groups. Surprisingly, even at baseline the HIV-positive women with advanced HIV-disease (Plow) had high BMI, with a median of 23.5, 48% of whom were overweight or obese (BMI >25 $kg/m^2$). Nref and Ppres had the same rates of overweight (65%) compared to Plow (44%). Ppres had the highest proportion of obese subjects (37%) compared to Nref (30%) and Plow (16%). Plow had smaller waist and hip circumferences than Nref and Ppres which were not different from each other. WHR was the same in Ppres and Plow (0.84) and greater than Nref (0.80), suggesting differences in distributions of adiposity between HIV-positive and HIV-negative women. Lean mass, fat mass, and fat:lean[2] were all lower in Plow while Nref and Ppres were not different. These patterns suggest that Nref and Ppres are more similar than Ppres and Plow and indicate that it is not appropriate to conflate all women with HIV infection as there are significant differences depending on the stage of disease. Usually lipodystrophy and alterations in body composition is attributed to ART exposure in the context of HIV infection. However, in this study the differences in body composition described at baseline were in ART-naïve individuals.

Weight in the Plow group increased by approximately 10% over the 12-month duration of the study and this increase was mostly due to the accrual of fat rather than lean mass. The high proportion of fat increase for a given increase in weight was unexpected; normally it would be anticipated that with weight gain the proportion of fat mass to lean was would be in the region of 55% to 45% respectively (Siervo *et al*, 2008). The women in the Plow group far exceeded these proportions; increases were 80% fat mass to 20% lean mass. A similar pattern was observed when HIV-positive women on ART were considered. Such a dramatic increase in adiposity in those with low $CD_4$ counts, 12

months after initiation of ART, is of concern and may be a risk factor for future metabolic disease. At baseline, Nref had smaller waist:hip ratios than Ppres and Plow. However, the relative stability of WHR over time suggests that fat is being deposited and not particularly on the hips or centrally. A WHR of >0.80 has been associated with cardio-metabolic risk, and is a more sensitive marker of central adiposity-induced pathology than BMI (Huxley *et al*, 2010; Coutinho *et al*, 2011). However, 2012 data from South Africa has suggested that the waist circumference used in Caucasian female populations (>80 cm) as part of the diagnosis of the metabolic syndrome is not appropriate for black South African women. Crowther *et al* suggest that the appropriate cut-off in this population is 91.5 cm and points to this as evidence of "a clear ethnic difference in the relationship between abdominal adiposity and metabolic disease risk" (Crowther *et al*, 2012). This observation serves as a reminder that it may not be appropriate to extrapolate Caucasian normative values onto black African populations with respect to body composition and measures of bone health. In a similar vein, a WHO report acknowledged that the "proportion of Asian people with a high risk of type 2 diabetes and cardiovascular disease is substantial at BMIs lower than the existing WHO cut-off point for overweight" (WHO Expert Consultation, 2004).

However, the burden of obesity in these women may contribute to increases in fracture risk as they age (Compston, 2013a; 2013b) although ongoing research is needed into the morbidity and mortality associated with fractures in obese patients, particularly in the developing world, the extent of which has not yet been defined.

## 9.2 Bone status

At baseline there were no differences in BMC, aBMD, or SA-BMC at any site between Nref, Ppres, and Plow. Differences in BA were not significant when adjusted for age, height, and weight. There were no differences in aBMD SDS at any skeletal site measured. These data are at variance with some published work but have the distinct advantage of having been derived from subjects prior to ART exposure and with the comparison of a HIV-negative control group.



Longitudinally, changes in BMC, aBMD, and SA-BMC were demonstrated over the 12-month duration of the study in Nref, Ppres, and Plow (see Table 9-1).

**Table 9-1 Changes in BMC, aBMD, & SA-BMC (Nref, Ppres, & Plow)**

| Site | | |
|---|---|---|
| Total hip | BMC | ↑ in all groups |
| | aBMD | ↑ Nref and Ppres |
| | SA-BMC | ↔ Nref and Ppres |
| | | ↓ **Plow (-1.7%)** |
| Femoral neck | BMC | ↔ in all groups |
| | aBMD | ↔ Nref and Ppres |
| | | ↓ **Plow (-2.4%)** |
| | SA-BMC | ↔ Nref and Ppres |
| | | ↓ **Plow (-2.7%)** |
| Lumbar Spine | BMC | ↔ Nref and Ppres |
| | | ↓ **Plow (-3.3%)** |
| | aBMD | ↑ Nref |
| | | ↓ **Plow (-2.0%)** |
| | SA-BMC | ↔ Nref and Ppres |
| | | ↓ **Plow (-2.1%)** |
| Whole body less head | BMC | ↔ in all groups |
| | aBMD | ↔ in all groups |
| | SA-BMC | ↔ in all groups |

Changes in BMC and SA-BMC from baseline and magnitude of changes.
↑, increase; ↓, decrease; ↔, no difference

There were decreases in aBMD at the femoral neck and lumbar spine, and SA-BMC at the hip, femoral neck and lumbar spine in Plow over the course of 12 months, i.e. after initiating ART. The magnitude of this decline reached -2.7 ±0.68% at the femoral neck. The analogous changes in the ART-unexposed and ART-exposed are shown in Table 9-2.

**Table 9-2 Changes in BMC, aBMD, & SA-BMC (ART-unexposed & ART-exposed)**

| Site | | | Comparison with Plow |
|---|---|---|---|
| Total hip | BMC | ↑ in both groups | |
| | aBMD | ↑ in both groups | = |
| | SA-BMC | ↓ **ART-exposed (-2.2%)** | > |
| Femoral neck | BMC | ↔ in both groups | |
| | aBMD | ↓ **ART-exposed (-2.3%)** | = |
| | SA-BMC | ↓ **ART-exposed (-2.7%)** | = |
| Lumbar Spine | BMC | ↓ **ART-exposed (-4.1%)** | > |
| | | ↔ in ART-unexposed | |
| | aBMD | ↓ **ART-exposed (-2.4%)** | > |
| | SA-BMC | ↓ **ART-exposed (-2.8%)** | > |
| | | ↔ in ART-unexposed | |
| Whole body less head | BMC | ↔ in both groups | |
| | aBMD | ↑ ART-unexposed | |
| | | ↓ ART-exposed | > |
| | SA-BMC | ↔ in both groups | |

Changes in BMC and SA-BMC from baseline, magnitude of changes and comparison with Plow.
↑, increase; ↓, decrease; ↔, no difference; >, greater than; =, equal to

The aBMD and SA-BMC changes, present in the *post hoc* analysis of ART exposure, ranged from -0.58 ±0.31% (WBLH) to -2.8 ±0.65% (lumbar spine) and exceed those (2%) used in the sample size calculation (see Section 3.5).

The importance of describing changes in BMC, aBMD and SA-BMC is due, in part, to the fact that the women are changing weight over the course of the study. This is particularly true in the Plow (and ART-exposed) subjects. BMC describes the actual mineral content changes over time and SA-BMC describes the changes in bone mineral after correction for weight. The change in aBMD will be between these values (BMC/BA). If, for example, bone mineral had increased in proportion to weight gain then BMC would have increased but SA-BMC would stay the same, again aBMD would be intermediate. If bone mineral had gone up less than expected for the amount of weight change, BMC would have increased but SA-BMC would decrease, aBMD would again be in-between.

At the hip, the latter pattern is evident in both Plow and ART-exposed suggesting less than expected increases in bone mineral for the amount of weight gained. At the femoral neck there were no changes in BMC but decreases in SA-BMC in Plow and ART-exposed,



suggesting an inappropriate increase in bone mineral for the amount of weight gained. At the lumbar spine there were significant decreases in BMC, aBMD, and SA-BMC in Plow and ART-exposed. It is feasible that that may have been even greater losses of bone mineral in Plow and ART-exposed had these losses not been partially compensated by increases in weight. There were no significant changes in BMC, aBMD or SA-BMC in Plow at WBLH although there were decreases in ART-exposed, and trends for SA-BMC reflected those in regions and the aBMD change at 12 months was significant.

These changes are important with respect to the magnitude of change; they compare with estimated aBMD annual loss of 0.5 – 1.0% in HIV-negative adults aged >35 years (Powderly *et al*, 2005) and 1 – 2% loss in the early menopause years (Hodgson *et al*, 2003). In postmenopausal women the OR for hip fracture is 2.6 for every SD decrease in aBMD from mean hip aBMD at age 30 years (Marshall *et al*, 1996), however it is not yet known if the changes described in this thesis will continue over time or plateau. It is also unknown if these decreases in bone mineral, in premenopausal women will translate into increased fracture risk either in the near future or following menopause.

The results presented in this thesis closely map the estimated rates of bone loss in Brown's ART study at 96 weeks, i.e. -2.3% and -2.5% for NNRTI and PI use respectively (Brown *et al*, 2009), a study of treatment-naïve patients commencing ART. It should be noted that the bone loss in Brown's study was over twice the time period compared to the study presented in this thesis. Duvivier *et al* describe a mean change in aBMD from baseline of -4.1 ±3.9% at 48 weeks at the LS and as great as -5.8 ±4.5% in those on PI-based ART (Duvivier *et al*, 2009) (see Section 2.4.3.3), which exceeds those seen in this study of South African women. The large increases in ALP activity in Plow and ART-exposed support the interpretation that bone loss is occurring in these groups and is accompanied by increased bone turnover (see Section 9.4).

There were unanticipated increases in BA at the total hip and, to a smaller degree, the femoral neck by original grouping and by *post hoc* ART exposure classification. This was

unanticipated because subjects were adult women who were unlikely to be undergoing bone expansion to this extent in 6 – 12 months. The fact that the same Hologic machine and software was used and operated by the same technician throughout this study with appropriate QC suggests that the results described are unlikely to be the result of technical differences, movement, positioning, or machine error. As speculated in Section 8.4, this is more likely to be due to a technical artefact because of overlying fat. The hip and femoral neck are the sites most likely to be affected by increases in gluteal and abdominal fat adjacent to, and overlying, the hip region (Tothill *et al*, 1997; Binkley *et al*, 2003).

There was particular loss of bone mineral at trabecular-rich sites such as the femoral neck and lumbar spine, compared to more cortically-rich WBLH. Trabecular rich sites are the most metabolically active, as a result of their high surface area, so it could be predicted that, in a situation analogous to bone loss related to pregnancy and lactation (Olausson *et al*, 2012), that ART-associated bone loss occurs predominantly at trabecular-rich sites. The majority of studies in HIV-positive patients suggest that the LS is the site of the greatest bone loss. This is probably because of the increased susceptibility of metabolically active trabecular bone to the effects of ART.

In the *post hoc* analysis there were decreases in BMC, aBMD, and SA-BMC at several skeletal sites in the ART-exposed group. There was increased urinary phosphate excretion and decreased tubular resorption of phosphate in ART-exposed also. This, in the context of good vitamin D status, and non-significantly different bone mineral status in HIV-positive women with high and low $CD_4$ counts when ART-naïve, supports the hypothesis that ART exposure (specifically tenofovir) is associated with loss of bone mineral over 12 months. In this study there is such overlap between Ppres and Plow, and ART-unexposed and ART-exposed that it was not possible to look at Plow and ART-unexposed (i.e. progressive HIV disease and bone loss in those with low $CD_4$ counts who did not receive ART). In this study premenopausal HIV-positive women with preserved $CD_4$ counts and HIV-negative controls demonstrated no evidence of bone loss, which is



in contrast to those HIV-positive women with low $CD_4$ counts who went on to be treated with ARTs.

In order to distinguish the specific effects of ART on bone health from other factors studies must: i) have a HIV-negative control group, and a HIV-positive group not exposed to ART against which to compare bone outcomes, ii) control for the 'traditional' osteoporosis risk factors (e.g. ethanol excess, poor vitamin D status) that are overrepresented in some HIV-positive cohorts, iii) involve cohorts large enough and with long enough duration of follow-up to determine both small changes in aBMD and clinical (namely fracture) outcome measures, and iv) allow for comparison of different ART drug combinations to look for differential effects on bone health. In such studies the "relative contributions of HIV infection, HIV viraemia and cART (combination-ART) to bone loss (which) remain poorly defined" will be better understood (Post *et al*, 2011). Such studies must also account for the relative difference in ages of the HIV-positive population and the population at large. While the HIV-positive population is ageing, it still remains relatively young, with a mean age several decades younger than the HIV-negative populations who develop osteoporosis and fracture their bones as a result of ageing. A germane observation is that even in individuals with relatively low peak bone mass, fragility fractures are uncommon before the age of 50 years. This is due, in part, to the probability that microarchitectural deterioration has yet to have developed (Raisz *et al*, 2008). For this reason it is important to observe HIV-positive individuals as they age to gather evidence for HIV and/or ART-associated bone loss and fracture.

## 9.3 Vitamin D status

At baseline there were no group differences in 25(OH)D concentrations with group mean values well in excess of 50nmol/l, although there was a tendency for there to be more Plow subjects with 25(OH)D concentrations <25nmol/l (5.3%), compared to 2.7% in Ppres and 1.0% in Nref.

Contrary to published findings there were no significant mean group differences in vitamin D status, at baseline or at 6 and 12 months with a similarly small percentage

with low 25(OH)D concentrations. The hypotheses that HIV-positive patients would have lower concentrations of 25(OH)D than controls and would demonstrate significantly greater declines in vitamin D status over time, particularly after the introduction of ART with its potent CYP450, hepatic enzyme inducing effects were not borne out. These data provide reassurance that, in 12 months, ART exposure did not lead to significant decreases in 25 (OH)D in this group of women living in Johannesburg.

Mean vitamin D status remained well above thresholds for risk of skeletal disease, including osteomalacia, in all groups throughout the study, and exceeded the IOM threshold of 50nmol/l deemed sufficient for bone health. The intriguing observation was that the Plow groups saw less marked wintertime decline in 25(OH)D compared with the other groups. It could be speculated that their relative lack of adiposity might mitigate against 25(OH)D sequestration into fat, however there was no significant relationship between 25(OH)D concentration and fat mass in ART-exposed subjects (p=0.75) and the pattern of blunted winter decline in Plow subjects disappeared when looking just at HIV-infected women (see Section 2.5).

In the *post hoc* analysis there was a greater increase in 25(OH)D in ART-unexposed compared to ART-exposed at 12 months. This may indicate a relative difference in vitamin D metabolism in those exposed to ART. The possibility of a 'relatively' poor vitamin D status in ART-exposed was supported by increases in ALP activity, although mean 25(OH)D concentrations were in the normal range and certainly higher than that usually associated with skeletal mineralisation defects (<25nmol/l). However without supporting histopathological evidence and more detailed evaluation of renal tubular function to exclude renal tubular acidosis as a cause a mineralisation deficit cannot be completely excluded. Other explanations for the differences in vitamin D status could include increased dermal synthesis of vitamin D (or greater dietary intake) in the non-exposed group. The combination of a raised ALP activity in the context of a relatively blunted increase in 25(OH)D in ART-exposed compared to ART-unexposed subjects may



suggest a perturbation in vitamin D metabolism that is too subtle to be reflected in gross serum 25(OH)D changes.

Vitamin D is increasingly recognised as being important for non-skeletal health outcomes, in particular the possible negative effect of poor vitamin D status on infectious disease susceptibility, progression, and outcomes (Holick, 2007). This requires further investigation particularly in populations at high risk of infectious diseases.

## 9.4 Mineral metabolism

In baseline biochemical variables there were non-significant group differences in serum creatinine, eGFR, and corrected calcium. There were significant, and predictable, differences in serum albumin with Plow having lower values than Nref and Ppres, and Ppres lower than Nref. Serum phosphate and TmP/GFR were greater in Plow than Nref and Ppres, and greater in Ppres than Nref. At baseline, TmP/GFR was higher in Plow (ART-exposed) compared to Ppres (ART-unexposed). By 12 months, Ppres appeared like Plow at baseline but Plow looked like Ppres at baseline. These observations may suggest that there were pre-existing differences in renal phosphate handling in HIV-infected women compared to controls and that ART exposure (or low $CD_4$ count) lowered renal thresholds for phosphate reabsorption.

Longitudinally, the alterations in phosphate metabolism with decreases in TmP/GFR of 11.2% in Plow and increases in Nref (6.4%) and Ppres (3.8%), coupled with increases in serum phosphate in Nref (7.0%) and Ppres (5.2%) but no change in Plow (0.0%) are in keeping with the a priori hypothesis of tenofovir-induced renal phosphate wasting and reflect the observations seen in other populations (Childs *et al*, 2008; Kinai *et al*, 2009; Bonjoch *et al*, 2012; Nishijima *et al*, 2012). It is plausible that the loss of bone mineral demonstrated in Plow and ART-exposed subjects was the result of renal phosphate wasting and subsequent leaching of phosphate from bone in order to maintain serum phosphate homeostasis.

The phosphate data support the observation that ART (specifically tenofovir) being associated with decreased bone mineral via urinary phosphate wasting when viewed in the context of tenofovir-exposure and a) decreased TmP/GFR, b) increased ALP, as a marker of osteoblast activity, and c) normal 25(OH)D concentrations. In the future it will be interesting to explore associations with PTH because of its positive associations with tenofovir exposure and with sodium chloride (salt) intakes. It is plausible that high salt intakes may have an additive effect on the kidney by promoting further phosphate loss in those exposed to tenofovir, as increased sodium intake results in decreased sodium and phosphate reabsorption in the proximal renal tubule.

It is noteworthy and as yet unexplained from the analyses conducted so far, why Plow and Ppres maintained higher serum phosphate concentrations than Nref. The expectation might be that HIV-positive subjects have lower serum phosphate concentrations than controls. The normal physiological range for serum phosphate is 0.87 – 1.45 mmol/l. These differences are marked and different between groups at each time point. At baseline the proportions of subjects with serum phosphate >1.4 mmol/l were 2%, 20%, and 27% in Nref, Ppres, and Plow respectively. At six months and 12 months these proportions were similar at 2%, 21%, and 28% (6 months) and 0%, 21%, and 25% (12 months) in Nref, Ppres, and Plow respectively. The proportions exceeding 1.4 mmol/l in Ppres and Plow greatly exceed the 2.5% expected from mean serum phosphate concentration +3 SD. Nref and Ppres are similar in most respects except for differences in serum phosphate and TmP/GFR. It suggests that Ppres may be intermediate between Nref and Plow in terms of renal phosphate handling and that this might act as a marker of future bone loss. However, this is speculative and remains to be seen during longer follow up studies. There are no published data describing hyperphosphataemia in HIV-positive ART-naïve subjects; indeed most reports of are hypophosphataemia in the context of ART exposure (Woodward *et al*, 2009). The mechanisms underlying these observations of higher serum phosphate in this group of women have yet to be elucidated but might include higher bone resorption and therefore lower PTH because



serum calcium would be raised resulting in supressed PTH concentrations to maintain serum calcium within the normal range. An alternative, or co-existing, mechanism could be a deficit in renal clearance of phosphate as a result of HIV infection.

The changes seen in serum albumin concentrations support established patterns seen in HIV infection and are in turn upheld by the demonstrated changes in weight. Lower serum albumin in HIV-positive patients is well described (Sudfeld *et al*, 2013) and is proportional to declining $CD_4$ count (Bhowmik *et al*, 2012). In this study, the Plow group had significantly lower albumin and weight at baseline. After the introduction of ART, and presumably improvements in appetite, and food intakes (Hendricks *et al*, 2006) accompanied by improvements in hepatic synthetic function and decreased catabolism seen with control of HIV viraemia, serum albumin concentrations increased in the Plow group over time. Likewise, serum albumin mean concentration in the ART-exposed group exceeded that in the ART-unexposed at 12 months. It is possible also that there were improvements in occult HIV-associated nephropathy (HIVAN) in those treated with ART. This glomerular disorder, with accompanying tubular and interstitial lesions, is associated with urinary albumin loss so would be improved by ART (Wyatt *et al*, 2012).

The measured increases in ALP activity in Plow over 12 months were for total ALP, however, given the change in bone mineral status it is likely to be of bone rather than hepatic origin, particularly when viewed in the context of phosphate results (see Section 7.1.5). However, this needs to be confirmed by measuring BALP in stored samples. It remains a possibility that the deleterious effects of ART exposure on bone mineral status could be a direct toxic effect of ART on bone, rather than the effect being mediated by renal phosphate loss. However, the balance of evidence would support the latter rather than the former explanation.

Plow could be demonstrating biochemical evidence of tenofovir induced renal injury (Calza, 2012; Maggi *et al*, 2012; Calza *et al*, 2013) and this is supported by the changes

seen in renal phosphate handling, which suggests a tubular pathology. Further investigation and more detailed investigation of renal function are warranted.

## 9.5 Participant retention

There was overall good retention in the study over the course of 12 months. Potential explanations for loss to follow-up were examined when possible (see Sections 6.2.1, 6.3.1). Anecdotally, retention may have been influenced by traditional beliefs that blood taken at the study visit is not replaced by the body. Other beliefs around blood were that it would be sold to others or would be used to transfuse to other people. The study team endeavoured to dispel these misconceptions by carefully explaining the purpose of the laboratory tests and allowing subjects to decline blood collection should they wish. These observations are anecdotal and require specific, qualitative investigation to examine the extent of these beliefs and the degree to which they affect willingness to enrol into clinically-based studies.

1.6% of participants who initially undertook to enrol in WBS then opted to join a different research study. This figure may well be an underestimate since this reason was only offered by those participants who actually explained why they did not wish to return for their follow-up visit. For subject safety reasons it was, in fact, a condition of taking part in the WBS, that participants were prohibited from taking part in another study (Table 3-2).

Whilst retention in the study was good, fewer subjects in Nref completed the study than Ppres or Plow. There are potential biases introduced by such non-response and non-attendance. These types of bias may limit the way in which data generated from this study are generalisable to other urban, black women residing in Soweto. In particular, the potential non-attendance bias might skew the overall results if, for example, the women who did not attend their follow-up visits were those with higher aBMD, then there may have been a greater difference in aBMD at 12 months than demonstrated in the study. It would appear from the distribution of non-attendance among the groups



that this was not a systematic effect but such sources of potential bias need to be borne in mind when interpreting the study results.

## 9.6 Future directions

Future directions for studies of HIV-associated bone mineral loss and changes in vitamin D status can be informed by some of the observations made from the data presented in this thesis and hypotheses generated as a result. These directions are divided into those that can be explored using existing data and stored samples and those that will require additional studies.

**Existing data and stored samples:**

1. HIV infection and ART may result in changes in bone geometry. These may be artefactual, such as that seen in increases in hip BA as a result of overlying fat in this study, or could potentially represent unanticipated periosteal apposition of bone in response to ART exposure. Analysis of serial pQCT scans will allow demonstration of changes in appendicular bone geometry and estimation of bone strength. Furthermore, pQCT may reveal insights into the associations between overweight and obesity and peripheral vascular calcification. The analysis of vertebral morphometry assessment (VMA) will allow investigation of the presence of spinal degeneration and undiagnosed spinal fractures.
2. The changes in body composition seen in this study warrant further exploration to examine why, and how, fat gain exceeds gains in lean tissue, and possibly bone, predicted from population studies. The use of DXA software to examine the distribution of fat in the participants (android versus gynaeoid, and peripheral versus central) may reveal insights into the change in risk of metabolic syndrome and obesity-related fracture. This work is being supported by a metabolic add-on study (Prof Shane Norris) to examine the effects of weight gain on metabolic and inflammatory markers.
3. I plan to further explore existing data and generate new laboratory data from stored biological samples and investigate these in the context of changes in body

seen in renal phosphate handling, which suggests a tubular pathology. Further investigation and more detailed investigation of renal function are warranted.

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**Existing data and stored samples:**

1. HIV infection and ART may result in changes in bone geometry. These may be artefactual, such as that seen in increases in hip BA as a result of overlying fat in this study, or could potentially represent unanticipated periosteal apposition of bone in response to ART exposure. Analysis of serial pQCT scans will allow demonstration of changes in appendicular bone geometry and estimation of bone strength. Furthermore, pQCT may reveal insights into the associations between overweight and obesity and peripheral vascular calcification. The analysis of vertebral morphometry assessment (VMA) will allow investigation of the presence of spinal degeneration and undiagnosed spinal fractures.
2. The changes in body composition seen in this study warrant further exploration to examine why, and how, fat gain exceeds gains in lean tissue, and possibly bone, predicted from population studies. The use of DXA software to examine the distribution of fat in the participants (android versus gynaeoid, and peripheral versus central) may reveal insights into the change in risk of metabolic syndrome and obesity-related fracture. This work is being supported by a metabolic add-on study (Prof Shane Norris) to examine the effects of weight gain on metabolic and inflammatory markers.
3. I plan to further explore existing data and generate new laboratory data from stored biological samples and investigate these in the context of changes in body

composition, bone mineral, vitamin D status, and mineral metabolism. These will include evaluation of PTH concentrations, bone turnover markers, adipokine concentrations, and urine markers of renal tubular function such as retinol-binding protein (RBP) and urine albumin/creatinine and protein/creatinine ratios. Another area of interest would be to explore genetic associations, in particular the rs2485209 single nucleotide polymorphism, which has been associated with changes in femoral neck BMC in black South African children (May *et al*, 2013). It would be interesting to explore the role of genetic determinants of HIV and ART-associated differences in bone mineral.

**Additional studies:**

1. ART exposure is associated with bone loss in HIV-positive black South African women. This may be due to direct effects of ART on bone, e.g. via impact on osteoclasts and indirectly through, for example, acid-base disturbance. This could be tested by detailed histomorphometric studies of bone tissue and detailed analysis of acid-base balance before and after the introduction of ART. A hypothesis underpinning the work presented in this thesis is that ART induces bone mineral to be preferentially lost from trabecular-rich sites because of increased metabolic activity in trabecular bone. Therefore, these sites are predisposed to the effects of ART-induced changes in mineral handling, the spine in particular may be a site vulnerable to rapid bone loss. This hypothesis could be evaluated further by examining changes in trabecular-rich sites using imaging techniques other than DXA, such as HRCT.
2. HIV infection is associated with lower 25(OH)D concentrations despite adequate opportunity for skin synthesis. Vitamin D may be synthesised less efficiently and/or consumed more rapidly in HIV-positives vs. controls. ART use may accelerate metabolism of available vitamin D in HIV-positive black South African women. This may be due to induction of hepatic and renal CYP450 enzyme pathways by drugs that impact on CYP450 pathways. This could be tested more



accurately by carrying out detailed vitamin D turnover studies (Jones *et al*, 2012) in HIV-positive patients and HIV-negative controls. Ideally, any vitamin D turnover study would be carried out before and after the introduction of ARTs.

3. In order to better understand the trajectory and magnitude of HIV- and ART-associated bone loss the study presented in this thesis (WBS) has been funded to be extended to 24 months and a further round of data collection will take place in April – May 2013. The aim of this extension is to ascertain if there is ongoing loss of bone after two years in the Plow/ART-exposed women and to compare with age-related changes and effects of body weight in HIV-uninfected controls, and to investigate the determinants of this. The clinical significance of the findings detailed in this study can be further explored by examination of BMD Z-scores (SDS) at the study endpoint. Whilst Z-scores in this group of premenopausal women have not been validated against future fracture risk because they were generated from South African data, they will be none-the-less interesting to evaluate, and may provide useful data regarding changes in aBMD within this population. In particular it will be interesting to see if the decrease in lumbar spine Z-score at 12 months will continue to decline at 24 months. Furthermore, it will allow me to chart further changes in body composition, particularly fat mass, and in renal function and markers of phosphate homeostasis. It will also provide evidence about changes in, or stability of, vitamin D status in those exposed to ART for 24 months.
4. As part of my post-doctoral research I plan to expand my research into the skeletal effects of other chronic infections such as hepatitis B, and their treatment. I have recently (March 2013) submitted a research proposal examining the effects of hepatitis B, and its treatment, on bone health to MRC Gambia Scientific Coordinating Committee (SCC) for consideration. I plan to continue to examine the role of HIV infection and ART exposure on bone mineral status and ideally develop a study to include clinical end points such as fracture data. Ideally, in future studies, there would be provision to undertake

histomorphological examination of bone tissue in patients before and after the introduction of ART and to use more sophisticated imaging techniques, such as high resolution (HR) pQCT to delineate, more precisely, the nature of bone loss and to compare this to DXA and biopsy results. Also, more sophisticated measures of renal tubular dysfunction, such as urinary concentrations of RBP could be employed to better isolate the precise location of renal insult (Campbell *et al*, 2012), and collection of 24-hour urinary collections would allow a more detailed analysis of renal mineral handling, and 2-hour timed urine collections to estimate renal phosphate excretion.

5. Another important area of research is to assess the effects of HIV infection and particularly ART exposure, at other times in the life course when women are vulnerable to mobilisation of skeletal mineral, such as during pregnancy and lactation. I have been working with Dr Gail Goldberg, Senior Investigator Scientist NBH, MRC HNR who has successfully secured funding for a Cambridge Gates PhD scholarship to investigate the effects of pregnancy and lactation on bone health in HIV-positive Ugandan women.
6. Another key life course event is the menopause transition. If HIV infection and ART exposure, and menopausal bone loss result in cumulative loss of bone mineral then it is possible that HIV-positive women in the early years following menopause could lose even more significant amounts of bone mineral, leaving them at higher risk of future fracture. This is particularly important because HIV infection is associated with premature ovarian failure and early menopause (Ohl *et al*, 2010; de Pommerol *et al*, 2011) which means that HIV-positive women may be relatively oestrogen deficient for significantly longer than their HIV-negative counterparts. The three subjects who probably were climacteric in WBS were in Plow (see Section 6.3.2) Likewise, HIV infection and ART exposure might augment the degree of pregnancy and lactational bone mineral loss and impair post-lactation gains in bone mineral. At present these are theoretical concerns but are important considerations when designing future studies of skeletal health



in HIV-positive women and those with other chronic infections such as hepatitis B and C.

7. Finally, if the two year data suggest ongoing bone loss in the face of renal phosphate wasting it would be interesting to address the utility of switching from tenofovir to, for example, abacavir to establish if this strategy would halt, or reverse, declines in bone demineralisation. An alternative study could assess the effect of oral phosphate replacement on bone mineralisation in the context of ongoing tenofovir exposure. In order to achieve this it is likely to require a sufficiently powered RCT with a prolonged duration of followup to demonstrate the effects of different ART on bone health.

## 9.7 Summary

Data generated from this study have demonstrated that there were no significant differences, at baseline, in three groups of women with respect to bone measures and vitamin D status. The differences in body composition (weight, BMI, fat mass, lean mass, fat:lean$^2$, and waist and hip circumferences) were all lower in Plow than Nref and Ppres, which were not significantly different from each other. At baseline only WHR was equivalent in Ppres and Plow, and greater than Nref, which might suggest that the proportions of adiposity were not changing.

This PhD has made some novel contributions to knowledge, these include the first observation of ART-associated bone loss, measured by DXA, in African women. WBS has added to the literature on tenofovir effects on renal function and bone health. It has demonstrated that ART exposure is associated with decreases in bone mineral and TmP/GFR, and whilst it cannot be described as causal it is likely to be related to tenofovir use. The study has also largely refuted the observations made in Western settings that HIV infection and ART exposure are associated with poor vitamin D status and that HIV infection with preserved $CD_4$ counts is associated with bone loss.

Preliminary results of this study have been presented as an oral presentation at the National Osteoporosis Foundation South Africa (NOFSA) meeting in Cape Town (April

2012) and as a poster presentation at the National Osteoporosis Society (NOS) meeting in Manchester (July 2012). A paper is in press describing the baseline body composition, bone and vitamin D findings (Appendix 7) and a further paper, on the baseline dietary data, is under review (Appendix 6).

In conclusion, the WBS has demonstrated that exposure to ART is associated with declines in bone mineral over time in urban, black South African women with immune compromise (as measured by $CD_4$ count) when compared with HIV-negative controls and HIV-positive patients not exposed to ART. These changes are apparent, and statistically significant, when the subjects are analysed by the groups established in the study design (Plow group) and when the ART-exposed subjects are examined in a *post hoc* analysis. The magnitude of this change, ≥2% over 12 months at the femoral neck and lumbar spine, exceeds that seen in early menopause and therefore is likely to be clinically significant. This study was not designed, or powered, to examine estimation of future fracture risk but the results do justify further study to assess if this rate of loss continues or, plateaus or reverses, as ongoing bone loss at this rate is likely to predispose to poor bone outcomes. The two-year follow-up study will examine if there is ongoing bone loss.

Unlike other studies in largely Caucasian populations, there were no significant differences in vitamin D status, and the hypothesis that ART use would result in up-regulation of vitamin D catabolism was not borne out although there are some subtle indications that exposure to ART may decrease winter declines in vitamin D status. However, there was no difference when split by ART-unexposed and ART-exposed. The ART-exposed 25(OH)D concentration were lower at 12 months and this observation requires longer duration of follow-up to ascertain longitudinal effects of ART exposure on vitamin D status.

This study has demonstrated how ART exposure is associated with perturbations in phosphate homeostasis and this supports the hypothesis that loss of bone mineral is



being driven by loss of phosphate by the kidney as a result of tenofovir toxicity at the proximal renal tubule.

The large (10%) increases in body fat in those women exposed to ART requires further exploration to see if this accumulation of adipose tissue continues and, if it does, what effect this will have on cardio-metabolic disease risk as well as on risk for increased rates of fracture.

As the global prevalence of HIV infection continues to rise and because infected patients are living long enough to develop age-related, non-communicable conditions, such as osteoporosis, it is likely that there will be a continued need to undertake studies of bone health in these populations in the future.

Much research is needed, not only to better understand the basic interrelationships between HIV, ART, body composition, bone health, and vitamin D status at an individual level but also to allow development of algorithms for prevention, treatment, and care pathways to be used in conjunction with those for screening for low aBMD and 25(OH)D in populations. Future research is required to ascertain, for example, how predictive low aBMD, measured by DXA, plasma 25(OH)D concentrations and calcium intakes are of future fragility fractures in Sub-Saharan African women with HIV infection.

## References:


Aberg JA. Aging, inflammation, and HIV infection. *Top Antivir Med* 2012;20:101-5.

Abrahamsen B. Patient level pooled analysis of 68 500 patients from seven major vitamin D fracture trials in US and Europe. *BMJ* 2010;340:b5463.

Adams JS, Hewison M. Unexpected actions of vitamin D: new perspectives on the regulation of innate and adaptive immunity. *Nat Clin Pract Endocrinol Metab* 2008;4:80-90.

Adams JS, Hewison M. Extrarenal expression of the 25-hydroxyvitamin D-1-hydroxylase. *Arch Biochem Biophys* 2012;523:95-102.

Alagarasu K, Selvaraj P, Swaminathan S, Narendran G, Narayanan PR. 5' regulatory and 3' untranslated region polymorphisms of vitamin D receptor gene in south Indian HIV and HIV-TB patients. *J Clin Immunol* 2009;29:196-204.

Allard JP, Aghdassi E, Chau J, Tam C, Kovacs CM, Salit IE, Walmsley SL. Effects of vitamin E and C supplementation on oxidative stress and viral load in HIV-infected subjects. *AIDS* 1998;12:1653-9.

Allison GT, Bostrom MP, Glesby MJ. Osteonecrosis in HIV disease: epidemiology, etiologies, and clinical management. *AIDS* 2003;17:1-9.

Altman DA. Comparing groups - continuous data. *Practical Statistics for Medical Research*. London: Chapman & Hall, 1999.

American College of Radiology, Radiological Society of North America. Patient Safety: Radiation dose in CT and X-ray exams. http://www.radiologyinfo.org/en/safety/index.cfm?pg=sfty_xray. (accessed January 2013).

Amiel C, Ostertag A, Slama L, Baudoin C, N'Guyen T, Lajeunie E, Neit-Ngeilh L, Rozenbaum W, De Vernejoul MC. BMD is reduced in HIV-infected men irrespective of treatment. *J Bone Miner Res* 2004;19:402-9.

Amorosa V, Tebas P. Bone disease and HIV infection. *Clin Infect Dis* 2006;42:108-14.

Anderson J, Garner S, Klemmer P. Diet, nutrients and bone health, 2012.

Anderson JJ, Pollitzer WS. Ethnic and genetic differences in susceptibility to osteoporotic fractures. *Adv Nutr Res* 1994;9:129-49.

Andriote J. *Victory deferred: How AIDS changed gay life in America*: University of Chicago Press, 1999.

Apostolova N, Blas-Garcia A, Esplugues JV. Mitochondrial interference by anti-HIV drugs: mechanisms beyond Pol-gamma inhibition. *Trends Pharmacol Sci* 2011;32:715-25.

Arnsten JH, Freeman R, Howard AA, Floris-Moore M, Santoro N, Schoenbaum EE. HIV infection and bone mineral density in middle-aged women. *Clin Infect Dis* 2006;42:1014-20.





Arnsten JH, Freeman R, Howard AA, Floris-Moore M, Lo Y, Klein RS. Decreased bone mineral density and increased fracture risk in aging men with or at risk for HIV infection. *AIDS* 2007;21:617-23.

Arpadi SM, McMahon D, Abrams EJ, Bamji M, Purswani M, Engelson ES, Horlick M, Shane E. Effect of bimonthly supplementation with oral cholecalciferol on serum 25-hydroxyvitamin D concentrations in HIV-infected children and adolescents. *Pediatrics* 2009;123:e121-6.

Arsenault JE, Aboud S, Manji KP, Fawzi WW, Villamor E. Vitamin supplementation increases risk of subclinical mastitis in HIV-infected women. *J Nutr* 2010;140:1788-92.

Aspray TJ, Prentice A, Cole TJ, Sawo Y, Reeve J, Francis RM. Low bone mineral content is common but osteoporotic fractures are rare in elderly rural Gambian women. *J Bone Miner Res* 1996;11:1019-25.

Aspray TJ, Yan L, Prentice A. Parathyroid hormone and rates of bone formation are raised in perimenopausal rural Gambian women. *Bone* 2005;36:710-20.

Aubin JE, Heersche JNM. Bone cell biology. Osteoblasts, osteocytes, and osteoclasts. In: Glorieux FH, ed. *Pediatric Bone*. London: Elsevier Science, 2002:43-77.

Aukrust P, Haug CJ, Ueland T, Lien E, Muller F, Espevik T, Bollerslev J, Froland SS. Decreased bone formative and enhanced resorptive markers in human immunodeficiency virus infection: indication of normalization of the bone-remodeling process during highly active antiretroviral therapy. *J Clin Endocrinol Metab* 1999;84:145-50.

Avenell A, MacLennan GS, Jenkinson DJ, McPherson GC, McDonald AM, Pant PR, Grant AM, Campbell MK, Anderson FH, Cooper C, Francis RM, Gillespie WJ *et al.* Long-term follow-up for mortality and cancer in a randomized placebo-controlled trial of vitamin D(3) and/or calcium (RECORD trial). *J Clin Endocrinol Metab* 2012;97:614-22.

'AVERT' website. http://www.avert.org/hiv-aids-history.htm. (accessed October 2012).

Bagga A, Bajpai A, Menon S. Approach to renal tubular disorders. *Indian J Pediatr* 2005;72:771-6.

Baum MK, Shor-Posner G, Lai S, Zhang G, Lai H, Fletcher MA, Sauberlich H, Page JB. High risk of HIV-related mortality is associated with selenium deficiency. *J Acquir Immune Defic Syndr Hum Retrovirol* 1997;15:370-4.

Beaupre LA, Jones CA, Johnston DW, Wilson DM, Majumdar SR. Recovery of function following a hip fracture in geriatric ambulatory persons living in nursing homes: prospective cohort study. *J Am Geriatr Soc* 2012;60:1268-73.

Bendavid E, Ford N, Mills EJ. HIV and Africa's elderly: the problems and possibilities. *AIDS* 2012;26 Suppl 1:S85-91.

Bhowmik A, Bhandari S, De R, Guha SK. Predictors of mortality among HIV-infected patients initiating anti retroviral therapy at a tertiary care hospital in Eastern India. *Asian Pac J Trop Med* 2012;5:986-90.

Bingham SA, Gill C, Welch A, Day K, Cassidy A, Khaw KT, Sneyd MJ, Key TJ, Roe L, Day NE. Comparison of dietary assessment methods in nutritional epidemiology: weighed records v. 24 h recalls, food-frequency questionnaires and estimated-diet records. *Br J Nutr* 1994;72:619-43.

Binkley N, Krueger D, Vallarta-Ast N. An overlying fat panniculus affects femur bone mass measurement. *J Clin Densitom* 2003;6:199-204.

Binkley N, Kiebzak GM, Lewiecki EM, Krueger D, Gangnon RE, Miller PD, Shepherd JA, Drezner MK. Recalculation of the NHANES database SD improves T-score agreement and reduces osteoporosis prevalence. *J Bone Miner Res* 2005;20:195-201.

Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am J Clin Nutr* 2006;84:18-28.

Bischoff-Ferrari HA, Willett WC, Orav EJ, Lips P, Meunier PJ, Lyons RA, Flicker L, Wark J, Jackson RD, Cauley JA, Meyer HE, Pfeifer M *et al.* A pooled analysis of vitamin D dose requirements for fracture prevention. *N Engl J Med* 2012;367:40-9.

Blackwood AM, Sagnella GA, Cook DG, Cappuccio FP. Urinary calcium excretion, sodium intake and blood pressure in a multi-ethnic population: results of the Wandsworth Heart and Stroke Study. *J Hum Hypertens* 2001;15:229-37.

Blake GM, Wahner HW, Folelman I. The Evaluation of Osteoporosis: dual energy X-ray absorptiometry and ultrasound in clinical practice. Edtion ed. In: Dunitz M, ed. London: Taylor & Francis, 1999.

Bolland MJ, Grey AB, Horne AM, Briggs SE, Thomas MG, Ellis-Pegler RB, Woodhouse AF, Gamble GD, Reid IR. Bone mineral density is not reduced in HIV-infected Caucasian men treated with highly active antiretroviral therapy. *Clin Endocrinol (Oxf)* 2006;65:191-7.

Bolland MJ, Grey A, Horne AM, Thomas MG. Osteomalacia in an HIV-infected man receiving rifabutin, a cytochrome P450 enzyme inducer: a case report. *Ann Clin Microbiol Antimicrob* 2008;7:3.

Bolland MJ, Wang TK, Grey A, Gamble GD, Reid IR. Stable bone density in HAART-treated individuals with HIV: A Metaanalysis. *J Clin Endocrinol Metab* 2011; 96:2721-31.

Bolland MJ, Grey A, Horne AM, Briggs SE, Thomas MG, Ellis-Pegler RB, Gamble GD, Reid IR. Effects of intravenous zoledronate on bone turnover and bone density persist for at least five years in HIV-infected men. J Clin Endocrinol Metab. 2012a;97:1922-8.

Bolland MJ, Grey A, Horne AM, Briggs SE, Thomas MG, Ellis-Pegler RB, Gamble GD, Reid IR. Stable bone mineral density over 6 years in HIV-infected men treated with highly active antiretroviral therapy (HAART). *Clin Endocrinol (Oxf)* 2012b;76:643-8.

Bonjoch A, Echeverria P, Perez-Alvarez N, Puig J, Estany C, Clotet B, Negredo E. High rate of reversibility of renal damage in a cohort of HIV-infected patients receiving tenofovir-containing antiretroviral therapy. *Antiviral Res* 2012;96:65-9.

Bonnick S, Lewis L. in Bonnick S and Lewis L, eds. An introduction to conventions in densometry. *Bone densitometry for technologists*: New York: Humana Press Inc, 2006:1-23.

Bor J, Herbst AJ, Newell ML, Bärnighausen T. Increases in adult life expectancy in rural South Africa: valuing the scale-up of HIV treatment. *Science* 2013;339:961-5.

Boufassa F, Goujard C, Viard JP, Carlier R, Lefebvre B, Yeni P, Bouchaud O, Capeau J, Meyer L, Vigouroux C, Group ACCS. Immune deficiency could be an early risk factor for altered insulin sensitivity in antiretroviral-naive HIV-1-infected patients: the ANRS COPANA cohort. *Antivir Ther* 2012;17:91-100.



Boulle A, Van Cutsem G, Hilderbrand K, Cragg C, Abrahams M, Mathee S, Ford N, Knight L, Osler M, Myers J, Goemaere E, Coetzee D *et al.* Seven-year experience of a primary care antiretroviral treatment programme in Khayelitsha, South Africa. *AIDS* 2010;24:563-72.

Brown T. Association between initiation of antiretroviral therapy with Efavirenz and decreases in 25-hydroxyvitamin D. 11th Intl Workshop on Adverse Drug Reactions. Philadelphia, 2009.

Brown TT, McComsey GA. Osteopenia and osteoporosis in patients with HIV: a review of current concepts. *Curr Infect Dis Rep* 2006a;8:162-70.

Brown TT, McComsey GA. Association between initiation of antiretroviral therapy with efavirenz and decreases in 25-hydroxyvitamin D. *Antivir Ther* 2010;15:425-9.

Brown TT, Qaqish RB. Antiretroviral therapy and the prevalence of osteopenia and osteoporosis: a meta-analytic review. *AIDS* 2006b;20:2165-74.

Brown TT, Ruppe MD, Kassner R, Kumar P, Kehoe T, Dobs AS, Timpone J. Reduced bone mineral density in human immunodeficiency virus-infected patients and its association with increased central adiposity and postload hyperglycemia. *J Clin Endocrinol Metab* 2004;89:1200-6.

Brown TT, McComsey GA, King MS, Qaqish RB, Bernstein BM, da Silva BA. Loss of bone mineral density after antiretroviral therapy initiation, independent of antiretroviral regimen. *J Acquir Immune Defic Syndr* 2009;51:554-61.

Bruera D, Luna N, David DO, Bergoglio LM, Zamudio J. Decreased bone mineral density in HIV-infected patients is independent of antiretroviral therapy. *AIDS* 2003;17:1917-23.

Bureau of Hygiene & Tropical Diseases. AIDS newsletter. 1986.

Burge R, Dawson-Hughes B, Solomon DH, Wong JB, King A, Tosteson A. Incidence and economic burden of osteoporosis-related fractures in the United States, 2005-2025. *J Bone Miner Res* 2007;22:465-75.

Calza L. Renal toxicity associated with antiretroviral therapy. *HIV Clin Trials* 2012;13:189-211.

Calza L, Trapani F, Salvadori C, Magistrelli E, Manfredi R, Colangeli V, Di Bari MA, Borderi M, Viale P. Incidence of renal toxicity in HIV-infected, antiretroviral-naïve patients starting tenofovir/emtricitabine associated with efavirenz, atazanavir/ritonavir, or lopinavir/ritonavir. *Scand J Infect Dis* 2013;45:147-54.

Campbell LJ, Dew T, Salota R, Cheserem E, Hamzah L, Ibrahim F, Sarafidis PA, Moniz CF, Hendry BM, Poulton M, Sherwood RA, Post FA. Total protein, albumin and low-molecular-weight protein excretion in HIV-positive patients. *BMC Nephrol* 2012;13:85.

Cantorna MT, Zhu Y, Froicu M, Wittke A. Vitamin D status, 1,25-dihydroxyvitamin D3, and the immune system. *Am J Clin Nutr* 2004;80:1717S-20S.

Carr A, Miller J, Eisman JA, Cooper DA. Osteopenia in HIV-infected men: association with asymptomatic lactic acidemia and lower weight pre-antiretroviral therapy. *AIDS* 2001;15:703-9.

Carvalho EH, Gelenske T, Bandeira F, Albuquerque Mda F. Bone mineral density in HIV-infected women taking antiretroviral therapy: a systematic review. *Arq Bras Endocrinol Metabol* 2010;54:133-42.

Castillo AB, Tarantal AF, Watnik MR, Martin RB. Tenofovir treatment at 30 mg/kg/day can inhibit cortical bone mineralization in growing rhesus monkeys (Macaca mulatta). *J Orthop Res* 2002;20:1185-9.

Cazanave C, Dupon M, Lavignolle-Aurillac V, Barthe N, Lawson-Ayayi S, Mehsen N, Mercie P, Morlat P, Thiebaut R, Dabis F. Reduced bone mineral density in HIV-infected patients: prevalence and associated factors. *AIDS* 2008;22:395-402.

Ceglia L, Harris SS. Vitamin D and its role in skeletal muscle. *Calcif Tissue Int* 2013;92:151-62.

Chan MY, Nguyen ND, Center JR, Eisman JA, Nguyen TV. Absolute fracture-risk prediction by a combination of calcaneal quantitative ultrasound and bone mineral density. *Calcif Tissue Int* 2012;90:128-36.

Chan MY, Nguyen ND, Center JR, Eisman JA, Nguyen TV. Quantitative ultrasound and fracture risk prediction in non-osteoporotic men and women as defined by WHO criteria. *Osteoporos Int* 2013;24:1015-22.

Chantler S, Dickie K, Goedecke JH, Levitt NS, Lambert EV, Evans J, Joffe Y, Micklesfield LK. Site-specific differences in bone mineral density in black and white premenopausal South African women. *Osteoporos Int* 2012;23:533-42.

Chapurlat RD, Meunier P. Bisphosphonates: a clinical perspective. In: Compston J, Ralston S, eds. *Osteoporosis and bone biology: The state of the art*. London: International Medical Press, 2000:75-88.

Chen D, Zhao M, Oyajobi B, Mundy GR. Update in bone cell biology. In: Compston J, Ralston S, eds. Osteoporosis and bone biology: The state of the art. London: International Medical Press, 2000:29-41.

Chigwedere P, Essex M. AIDS denialism and public health practice. *AIDS Behav* 2010;14:237-47.

Chigwedere P, Seage GR, Gruskin S, Lee TH, Essex M. Estimating the lost benefits of antiretroviral drug use in South Africa. *J Acquir Immune Defic Syndr* 2008;49:410-5.

Childs K, Fishman S, Bateman K. Should vitamin D be prescribed with tenofovir/FTC? 48th International Conference on Antimicrobial Agents and Chemotherapy. Washington, DC, 2008.

Childs K, Fishman S, Factor S, Dieterich D, Mullen M, Branch A. First report of dose/response data of HIV-infected men treated with Vitamin D3 supplements. CROI. Montreal, 2009.

Childs K, Welz T, Samarawickrama A, Post FA. Effects of vitamin D deficiency and combination antiretroviral therapy on bone in HIV-positive patients. *AIDS* 2012;26:253-62.

Chisholm D, Baltussen R, Evans DB, Ginsberg G, Lauer JA, Lim S, Ortegon M, Salomon J, Stanciole A, Edejer TT. What are the priorities for prevention and control of non-communicable diseases and injuries in sub-Saharan Africa and South East Asia? *BMJ* 2012;344:e586.



Clay PG, Voss LE, Williams C, Daume EC. Valid treatment options for osteoporosis and osteopenia in HIV-infected persons. *Ann Pharmacother* 2008;42:670-9.

Cole TJ. Sympercents: symmetric percentage differences on the 100 log(e) scale simplify the presentation of log transformed data. *Stat Med* 2000;19:3109-25.

Cole TJ, Prentice A. Bone mineral measurements. *BMJ* 1992;305:1223-4.

Collin F, Duval X, Le Moing V, Piroth L, Al Kaied F, Massip P, Villes V, Chene G, Raffi F. Ten-year incidence and risk factors of bone fractures in a cohort of treated HIV1-infected adults. *AIDS* 2009;23:1021-4.

Compston J. The pathogenesis of osteoporosis. In: Arden NK, Spector TD, eds. *Osteoporosis illustrated*. London: Current Medical Literature Ltd, 1997:17-35.

Compston JE. The pathogenesis and management of osteoporosis. In: Compston JE, Ralston S, eds. *Osteoporosis and bone biology: The state of the art*. London: International Medical Press, 2000:59-73.

Compston J. Obesity and bone. *Curr Osteoporos Rep* 2013a;11:30-5.

Compston J. Obesity and fractures. *Joint Bone Spine* 2013b;80:8-10.

Compston J, Cooper A, Cooper C, Francis R, Kanis JA, Marsh D, McCloskey EV, Reid DM, Selby P, Wilkins M, (NOGG) NOGG. Guidelines for the diagnosis and management of osteoporosis in postmenopausal women and men from the age of 50 years in the UK. *Maturitas* 2009;62:105-8.

Compston JE, Watts NB, Chapurlat R, Cooper C, Boonen S, Greenspan S, Pfeilschifter J, Silverman S, Díez-Pérez A, Lindsay R, Saag KG, Netelenbos JC *et al.* Obesity is not protective against fracture in postmenopausal women: GLOW. *Am J Med* 2011;124:1043-50.

Conesa-Botella A, Florence E, Lynen L, Colebunders R, Menten J, Moreno-Reyes R. Decrease of vitamin D concentration in patients with HIV infection on a non nucleoside reverse transcriptase inhibitor-containing regimen. *AIDS Res Ther* 2010;7:40.

Conesa-Botella A, Meintjes G, Coussens AK, van der Plas H, Goliath R, Schutz C, Moreno-Reyes R, Mehta M, Martineau AR, Wilkinson RJ, Colebunders R, Wilkinson KA. Corticosteroid therapy, vitamin D status, and inflammatory cytokine profile in the HIV-tuberculosis immune reconstitution inflammatory syndrome. *Clin Infect Dis* 2012;55:1004-11.

Consensus Development Conference. Prophylaxis and treatment of osteoporosis. *Osteoporos Int* 1991;1:114-7.

Cournil A, Eymard-Duvernay S, Diouf A, Moquet C, Coutherut J, Ngom Gueye NF, Cames C, Taverne B, Bork K, Sow PS, Delaporte E, Group AS. Reduced quantitative ultrasound bone mineral density in HIV-infected patients on antiretroviral therapy in Senegal. *PLoS One* 2012;7:e31726.

Coussens AK, Wilkinson RJ, Hanifa Y, Nikolayevskyy V, Elkington PT, Islam K, Timms PM, Venton TR, Bothamley GH, Packe GE, Darmalingam M, Davidson RN *et al.* Vitamin D accelerates resolution of inflammatory responses during tuberculosis treatment. *Proc Natl Acad Sci USA* 2012;109:15449-54.

Coutinho T, Goel K, Corrêa de Sá D, Kragelund C, Kanaya AM, Zeller M, Park JS, Kober L, Torp-Pedersen C, Cottin Y, Lorgis L, Lee SH *et al.* Central obesity and survival in subjects with coronary artery disease: a systematic review of the literature and collaborative analysis with individual subject data. *J Am Coll Cardiol* 2011;57:1877-86.

Coutsoudis A, Bobat RA, Coovadia HM, Kuhn L, Tsai WY, Stein ZA. The effects of vitamin A supplementation on the morbidity of children born to HIV-infected women. *Am J Public Health* 1995;85:1076-81.

Cozzolino M, Vidal M, Arcidiacono MV, Tebas P, Yarasheski KE, Dusso AS. HIV-protease inhibitors impair vitamin D bioactivation to 1,25-dihydroxyvitamin D. *AIDS* 2003;17:513-20.

Croucher PI, Garrahan NJ, Compston JE. Assessment of cancellous bone structure: comparison of strut analysis, trabecular bone pattern factor, and marrow space star volume. *J Bone Miner Res* 1996;11:955-61.

Crowther NJ, Norris SA. The current waist circumference cut point used for the diagnosis of metabolic syndrome in sub-Saharan African women is not appropriate. *PLoS One* 2012;7:e48883.

Dao CN, Patel P, Overton ET, Rhame F, Pals SL, Johnson C, Bush T, Brooks JT. Low vitamin D among HIV-infected adults: prevalence of and risk factors for low vitamin D levels in a cohort of HIV-infected adults and comparison to prevalence among adults in the US general population. *Clin Infect Dis* 2011;52:396-405.

Department of Health. The Ionising Radiation (Medical Exposure) Regulations 2000. http://www.dh.gov.uk/prod_consum_dh/groups/dh_digitalassets/@dh/@en/documents/digitalasset/dh_064707.pdf. (accessed June 2012).

van Deventer HE, George JA, Paiker JE, Becker PJ, Katz IJ. Estimating glomerular filtration rate in black South Africans by use of the modification of diet in renal disease and Cockcroft-Gault equations. *Clin Chem* 2008;54:1197-202.

DIPART (Vitamin D Individual Patient Analysis of Randomized Trials) Group. Patient level pooled analysis of 68 500 patients from seven major vitamin D fracture trials in US and Europe. *BMJ* 2010;340:b5463.

Dolan SE, Kanter JR, Grinspoon S. Longitudinal analysis of bone density in human immunodeficiency virus-infected women. *J Clin Endocrinol Metab* 2006;91:2938-45.

Dowd DR, MacDonald PN. The molecular biology of the vitamin D receptor. In: Holick MF, ed. *Vitamin D physiology, molecular biology and clinical applications*. New York: Humana Press Inc, 2010:135-52.

Drezner M. Phosphorus homeostasis and related disorders. In: Bilezikian JP, Raisz, LG, Martin TJ, eds. *Principles of bone biology*. San Diego: Academic Press, 2008:465-87.

Drincic AT, Armas LA, Van Diest EE, Heaney RP. Volumetric dilution, rather than sequestration best explains the low vitamin D status of obesity. *Obesity (Silver Spring)* 2012;20:1444-8.

Dube MP, Parker RA, Mulligan K, Tebas P, Robbins GK, Roubenoff R, Grinspoon SK. Effects of potent antiretroviral therapy on free testosterone levels and fat-free mass in men in a prospective, randomized trial: A5005s, a substudy of AIDS Clinical Trials Group Study 384. *Clin Infect Dis* 2007;45:120-6.



Duvivier C, Kolta S, Assoumou L, Ghosn J, Rozenberg S, Murphy RL, Katlama C, Costagliola D. Greater decrease in bone mineral density with protease inhibitor regimens compared with nonnucleoside reverse transcriptase inhibitor regimens in HIV-1 infected naive patients. *AIDS* 2009;23:817-24.

Effros RB, Fletcher CV, Gebo K, Halter JB, Hazzard WR, Horne FM, Huebner RE, Janoff EN, Justice AC, Kuritzkes D, Nayfield SG, Plaeger SF *et al.* Aging and infectious diseases: workshop on HIV infection and aging: what is known and future research directions. *Clin Infect Dis* 2008;47:542-53.

Fabbriciani G, De Socio GV, Massarotti M. Antiretroviral therapy and adverse skeletal effects. *Mayo Clin Proc* 2011;86:916-7; author reply 7.

Fakruddin JM, Laurence J. HIV envelope gp120-mediated regulation of osteoclastogenesis via receptor activator of nuclear factor kappa B ligand (RANKL) secretion and its modulation by certain HIV protease inhibitors through interferon-gamma/RANKL cross-talk. *J Biol Chem* 2003;278:48251-8.

Fausto A, Bongiovanni M, Cicconi P, Menicagli L, Ligabo EV, Melzi S, Bini T, Sardanelli F, Cornalba G, Monforte A. Potential predictive factors of osteoporosis in HIV-positive subjects. *Bone* 2006;38:893-7.

Fawzi WW, Msamanga GI, Spiegelman D, Wei R, Kapiga S, Villamor E, Mwakagile D, Mugusi F, Hertzmark E, Essex M, Hunter DJ. A randomized trial of multivitamin supplements and HIV disease progression and mortality. *N Engl J Med* 2004;351:23-32.

Fenton TR, Tough SC, Lyon AW, Eliasziw M, Hanley DA. Causal assessment of dietary acid load and bone disease: a systematic review & meta-analysis applying Hill's epidemiologic criteria for causality. *Nutr J* 2011;10:41.

Fernandez-Fernandez B, Montoya-Ferrer A, Sanz AB, Sanchez-Niño MD, Izquierdo MC, Poveda J *et al*. Tenofovir nephrotoxicity: 2011 update. *AIDS Res Treat*. 2011; 2011: 354908

Fernandez-Rivera J, Garcia R, Lozano F, Macias J, Garcia-Garcia JA, Mira JA, Corzo JE, Gomez-Mateos J, Rueda A, Sanchez-Burson J, Pineda JA. Relationship between low bone mineral density and highly active antiretroviral therapy including protease inhibitors in HIV-infected patients. *HIV Clin Trials* 2003;4:337-46.

Ferrari S, Bianchi ML, Eisman JA, Foldes AJ, Adami S, Wahl DA, Stepan JJ, de Vernejoul MC, Kaufman JM. Osteoporosis in young adults: pathophysiology, diagnosis, and management. *Osteoporos Int* 2012;23:2735-48.

Fihlani P. 'Whoonga' threat to South African HIV patients. http://www.bbc.co.uk/news/world-africa-12389399. (accessed March 2013).

Fischl MA, Richman DD, Grieco MH, Gottlieb MS, Volberding PA, Laskin OL, Leedom JM, Groopman JE, Mildvan D, Schooley RT. The efficacy of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo-controlled trial. *N Engl J Med* 1987;317:185-91.

Forsyth SF, Gazzard BG, Nelson MR. A case of march fracture in a patient with osteoporosis on long-term HAART. *Int J STD AIDS* 2002;13:645-6.

National Kidney Foundation. Frequently asked questions about GFR estimates. National Kidney Foundation 2011:19.

Gallant JE, Staszewski S, Pozniak AL, DeJesus E, Suleiman JM, Miller MD, Coakley DF, Lu B, Toole JJ, Cheng AK. Efficacy and safety of tenofovir DF vs stavudine in combination therapy in antiretroviral-naive patients: a 3-year randomized trial. *JAMA* 2004;292:191-201.

Gauthier A, Kanis JA, Jiang Y, Martin M, Compston JE, Borgström F, Cooper C, McCloskey EV. Epidemiological burden of postmenopausal osteoporosis in the UK from 2010 to 2021: estimations from a disease model. *Arch Osteoporos* 2011;6:179-88.

Gibellini D, De Crignis E, Ponti C, Cimatti L, Borderi M, Tschon M, Giardino R, Re MC. HIV-1 triggers apoptosis in primary osteoblasts and HOBIT cells through TNFalpha activation. *J Med Virol* 2008;80:1507-14.

Gillespie LD, Robertson MC, Gillespie WJ, Sherrington C, Gates S, Clemson LM, Lamb SE. Interventions for preventing falls in older people living in the community. *Cochrane Database Syst Rev* 2012;9:CD007146.

Glasgow University. Functional Anantomy and Biomechanics. http://www.gla.ac.uk/t4/~fbls/files/fab/tutorial/clinical/orhip.html. (accessed April 2013).

Global Health Council. Needs and costs (HIV expenditure). http://www.globalhealth.org/hiv_aids/needs/. (accessed May 2010).

Glüer CC. Monitoring skeletal changes by radiological techniques. *J Bone Miner Res* 1999;14:1952-62.

Goedecke JH, Micklesfield LK, Levitt NS, Lambert EV, West S, Maartens G, Dave JA. Effect of different antiretroviral drug regimens on body fat distribution of HIV-infected South African women. *AIDS Res Hum Retroviruses* 2013;29:557-63.

Gold J, Pocock N, Li Y. Bone mineral density abnormalities in patients with HIV infection. *J Acquir Immune Defic Syndr* 2002;30:131-2.

Goldring SR, Goldring MB. Eating bone or adding it: the Wnt pathway decides. *Nat Med* 2007;13:133-4.

Goulet JL, Fultz SL, Rimland D, Butt A, Gibert C, Rodriguez-Barradas M, Bryant K, Justice AC. Aging and infectious diseases: do patterns of comorbidity vary by HIV status, age, and HIV severity? *Clin Infect Dis* 2007;45:1593-601.

Gounder CR, Wada NI, Kensler C, Violari A, McIntyre J, Chaisson RE, Martinson NA. Active tuberculosis case-finding among pregnant women presenting to antenatal clinics in Soweto, South Africa. *J Acquir Immune Defic Syndr* 2011;57:e77-84.

Grabowski P. Physiology of bone. In: Allgrove J, Shaw N, eds. *Calcium and bone disorders in children and adolescents*. Basel Switzerland: Karger, 2009:8-49.

Grant AM, Avenell A, Campbell MK, McDonald AM, MacLennan GS, McPherson GC, Anderson FH, Cooper C, Francis RM, Donaldson C, Gillespie WJ, Robinson CM *et al.* Oral vitamin D3 and calcium for secondary prevention of low-trauma fractures in elderly people (Randomised Evaluation of Calcium Or vitamin D, RECORD): a randomised placebo-controlled trial. *Lancet* 2005;365:1621-8.

Griffiths PL, Sheppard ZA, Johnson W, Cameron N, Pettifor JM, Norris SA. Associations between household and neighbourhood socioeconomic status and systolic blood pressure among urban South African adolescents. *J Biosoc Sci* 2012;44:433-58.



Gross H. http://upload.wikimedia.org/wikipedia/commons/f/f3/Hiv_gross.png. (accessed May 2012).

Grund B, Peng G, Gibert CL, Hoy JF, Isaksson RL, Shlay JC, Martinez E, Reiss P, Visnegarwala F, Carr AD. Continuous antiretroviral therapy decreases bone mineral density. *AIDS* 2009;23:1519-29.

Guaraldi G, Ventura P, Albuzza M, Orlando G, Bedini A, Amorico G, Esposito R. Pathological fractures in AIDS patients with osteopenia and osteoporosis induced by antiretroviral therapy. *AIDS* 2001;15:137-8.

Guateng: City of Johannesburg. Region D. http://www.joburg.org.za/index.php?option=com_content&do_pdf=1&id=174&limitstart=1. (accessed February 2013).

Gullberg B, Johnell O, Kanis JA. World-wide projections for hip fracture. *Osteoporos Int* 1997;7:407-13.

Gupta RP, Hollis BW, Patel SB, Patrick KS, Bell NH. CYP3A4 is a human microsomal vitamin D 25-hydroxylase. *J Bone Miner Res* 2004;19:680-8.

Gyllensten K, Josephson F, Lidman K, Saaf M. Severe vitamin D deficiency diagnosed after introduction of antiretroviral therapy including efavirenz in a patient living at latitude 59 degrees N. *AIDS* 2006;20:1906-7.

Hall V, Thomsen RW, Henriksen O, Lohse N. Diabetes in Sub Saharan Africa 1999-2011: epidemiology and public health implications: A systematic review. *BMC Public Health* 2011;11:564.

Hanrahan CF, Golub JE, Mohapi L, Tshabangu N, Modisenyane T, Chaisson RE, Gray GE, McIntyre JA, Martinson NA. Body mass index and risk of tuberculosis and death: a prospective cohort of HIV-infected adults from South Africa. *AIDS (London, England)* 2010 19;24:1501-8.

Hansen AB, Obel N, Nielsen H, Pedersen C, Gerstoft J. Bone mineral density changes in protease inhibitor-sparing vs. nucleoside reverse transcriptase inhibitor-sparing highly active antiretroviral therapy: data from a randomized trial. *HIV Med* 2011;12:157-65.

Harvey N, Dennison E, Cooper C. Osteoporosis: impact on health and economics. *Nat Rev Rheumatol* 2010;6:99-105.

Hasserius R, Karlsson MK, Jónsson B, Redlund-Johnell I, Johnell O. Long-term morbidity and mortality after a clinically diagnosed vertebral fracture in the elderly – a 12- and 22-year follow-up of 257 patients. *Calcif Tissue Int* 2005;76:235-42.

Haug C, Muller F, Aukrust P, Froland SS. Subnormal serum concentration of 1,25-vitamin D in human immunodeficiency virus infection: correlation with degree of immune deficiency and survival. *J Infect Dis* 1994;169:889-93.

Heaney RP, Holick MF. Why the IOM recommendations for vitamin D are deficient. *J Bone Miner Res* 2011;26:455-7.

Hecht R, Bollinger L, Stover J, McGreevey W, Muhib F, Madavo CE, de Ferranti D. Critical choices in financing the response to the global HIV/AIDS pandemic. *Health Aff (Millwood)* 2009;28:1591-605.

Hellman P, Albert J, Gidlund M, Klareskog L, Rastad J, Akerstrom G, Juhlin C. Impaired parathyroid hormone release in human immunodeficiency virus infection. *AIDS research and human retroviruses* 1994;10:391-4.

Hendricks KM, Willis K, Houser R, Jones CY. Obesity in HIV infection: dietary correlates. *J Am Coll Nutr* 2006;25:321-31.

Hillier S, Cooper C. The epidemiology of osteoporosis. In: Arden NK, Spector TD, eds. *Osteoporosis illustrated*. London: Current Medical Literature Ltd, 1997:1-13.

Hodgson S. American association of clinical endocrinologists medical guidelines for clinical practice for the prevention and treatment of postmenopausal osteoporosis: 2001 edition, with selected updates for 2003*. http://www.aace.com/pub/pdf/guidelines/osteoporosis2001Revised.pdf. (accessed July 2012).

Holick MF. High prevalence of vitamin D inadequacy and implications for health. *Mayo Clin Proc* 2006;81:353-73.

Holick MF. Vitamin D deficiency. *N Engl J Med* 2007;357:266-81.

Holick MF. Deficiency of sunlight and vitamin D. *BMJ* 2008;336:1318-9.

Holick M. The vitamin D deficiency pandemic: a forgotten hormone important for health. Public Health Reviews 2010; Vol. 32, No 1:267-83. http://www.publichealthreviews.eu/upload/pdf_files/7/15_Vitamin_D.pdf. (accessed July 2012).

Holick MF. The IOM D-lemma. *Public Health Nutr* 2011;14:939-41.

Hontelez JA, de Vlas SJ, Baltussen R, Newell ML, Bakker R, Tanser F, Lurie M, Bärnighausen T. The impact of antiretroviral treatment on the age composition of the HIV epidemic in sub-Saharan Africa. *AIDS* 2012;26 Suppl 1:S19-30.

Hough S. *NOFSA guidelines for the diagnosis and management of osteoporosis*. South Africa: Journal of Endocrinology , Metabolism and Diabetes of South Africa, 2010.

Hoy J. Bone, fracture and frailty. *Curr Opin HIV AIDS* 2011; 6:309-14.

Huh SY, Gordon CM. Vitamin D deficiency in children and adolescents: epidemiology, impact and treatment. *Rev Endocr Metab Disord* 2008;9:161-70.

Hurley E, Coutsoudis A, Giddy J, Knight SE, Loots E, Esterhuizen TM. Weight evolution and perceptions of adults living with HIV following initiation of antiretroviral therapy in a South African urban setting. *S Afr Med J* 2011;101:645-50.

Huxley R, Mendis S, Zheleznyakov E, Reddy S, Chan J. Body mass index, waist circumference and waist:hip ratio as predictors of cardiovascular risk – a review of the literature. *Eur J Clin Nutr* 2010;64:16-22.

Innes S, Cotton MF, Haubrich R, Conradie MM, van Niekerk M, Edson C, Rabie H, Jain S, Sun X, Zöllner W, Hough S, Browne SH. High prevalence of lipoatrophy in pre-pubertal South African children on antiretroviral therapy: a cross-sectional study. *BMC Pediatr* 2012;12:183.



Institute of Medicine. Dietary Reference Intakes: Elements. http://www.iom.edu/Home/Global/News%20Announcements/~/media/Files/Activity%20Files/Nutrition/DRIs/DRI_Elements.ashx. (accessed April 2013).

Institute of Medicine. Dietary reference intakes for calcium and vitamin D: The National Academies Press, 2010.

International Atomic Energy Agency. Dual energy X ray absorpiometry for bone mineral density and body composition assessment. Vienna: IAEA, 2010.

IOF. Facts and statistics about osteoporosis and its impact. http://www.iofbonehealth.org/facts-and-statistics.html. (accessed May 2010).

Isanaka S, Mugusi F, Hawkins C, Spiegelman D, Okuma J, Aboud S, Guerino C, Fawzi WW. Effect of high-dose vs standard-dose multivitamin supplementation at the initiation of HAART on HIV disease progression and mortality in Tanzania: a randomized controlled trial. *JAMA* 2012;308:1535-44.

Jacobsen SJ, Goldberg J, Miles TP, Brody JA, Stiers W, Rimm AA. Regional variation in the incidence of hip fracture. US white women aged 65 years and older. *JAMA* 1990;264:500-2.

Jacobson DL, Bica I, Knox TA, Wanke C, Tchetgen E, Spiegelman D, Silva M, Gorbach S, Wilson IB. Difficulty swallowing and lack of receipt of highly active antiretroviral therapy predict acute weight loss in human immunodeficiency virus disease. *Clin Infect Dis* 2003;37:1349-56.

Jain RG, Lenhard JM. Select HIV protease inhibitors alter bone and fat metabolism ex vivo. *J Biol Chem* 2002;277:19247-50.

Jarjou LM, Laskey MA, Sawo Y, Goldberg GR, Cole TJ, Prentice A. Effect of calcium supplementation in pregnancy on maternal bone outcomes in women with a low calcium intake. *Am J Clin Nutr* 2010;92:450-7.

Jones KS, Schoenmakers I, Bluck LJ, Ding S, Prentice A. Plasma appearance and disappearance of an oral dose of 25-hydroxyvitamin D2 in healthy adults. *Br J Nutr* 2012;107:1128-37.

Jones S, Restrepo D, Kasowitz A, Korenstein D, Wallenstein S, Schneider A, Keller MJ. Risk factors for decreased bone density and effects of HIV on bone in the elderly. *Osteoporos Int* 2008;19:913-8.

Kanis JA, Hans D, Cooper C, Baim S, Bilezikian JP, Binkley N, Cauley JA, Compston JE, Dawson-Hughes B, El-Hajj Fuleihan G, Johansson H, Leslie WD *et al.* Interpretation and use of FRAX in clinical practice. *Osteoporos Int* 2011;22:2395-411.

Kanis JA, McCloskey E, Johansson H, Oden A, Leslie WD. FRAX(®) with and without bone mineral density. *Calcif Tissue Int* 2012;90:1-13.

Khaw KT, Reeve J, Luben R, Bingham S, Welch A, Wareham N, Oakes S, Day N. Prediction of total and hip fracture risk in men and women by quantitative ultrasound of the calcaneus: EPIC-Norfolk prospective population study. *Lancet* 2004;363:197-202.

Khoo AL, Chai L, Koenen H, Joosten I, Netea M, van der Ven A. Translating the role of vitamin D3 in infectious diseases. *Crit Rev Microbiol* 2012;38:122-35.

Kiebzak GM, Binkley N, Lewiecki EM, Miller PD. Diagnostic agreement at the total hip using different DXA systems and the NHANES III database. *J Clin Densitom* 2007;10:132-7.

Kinai E, Hanabusa H. Progressive renal tubular dysfunction associated with long-term use of tenofovir DF. *AIDS Res Hum Retroviruses* 2009;25:387-94.

Kirkwood BR, Sterne JAC. Comparison of means from several groups: analysis of variance. In: Kirkwood BR, Sterne JAC, eds. *Medical statistics*. Malden: Blackwell Publishing Company, 2005:80-86.

Klein-Nulend J, Bonewald LF. The Osteocyte. In: Bilezikian JP, Raisz LG, Martin TJ, eds. *Principles of Bone Biology*. USA: Academic Press, 2008:153-74.

Klemmer PJ, Anderson JJB. Renal regulation of calcium and phosphate Ions. In: Anderson JJB, Garner SC, Klemmer PJ, eds. *Diet, Nutrients and Bone Health*. Boca Raton: CRC Press, 2012:113-120.

Kruger MC, Kruger IM, Wentzel-Viljoen E, Kruger A. Urbanization of black South African women may increase risk of low bone mass due to low vitamin D status, low calcium intake, and high bone turnover. *Nutr Res* 2011;31:748-58.

Kwan CK, Eckhardt B, Baghdadi J, Aberg JA. Hyperparathyroidism and complications associated with vitamin D deficiency in HIV-Infected adults in New York City, New York. *AIDS Res Hum Retroviruses* 2012;28:825-32.

Labadarios D, Steyn NP, Maunder E, MacIntryre U, Gericke G, Swart R, Huskisson J, Dannhauser A, Vorster HH, Nesmvuni AE, Nel JH. The National Food Consumption Survey (NFCS): South Africa, 1999. *Public Health Nutr* 2005;8:533-43.

Laskey MA, de Bono S, Zhu D, Shaw CN, Laskey PJ, Ward KA, Prentice A. Evidence for enhanced characterization of cortical bone using novel pQCT shape software. *J Clin Densitom* 2010;13:247-55.

Lemey P, Pybus OG, Wang B, Saksena NK, Salemi M, Vandamme AM. Tracing the origin and history of the HIV-2 epidemic. *Proc Natl Acad Sci USA* 2003;100:6588-92.

Lenders CM, Feldman HA, Von Scheven E, Merewood A, Sweeney C, Wilson DM, Lee PD, Abrams SH, Gitelman SE, Wertz MS, Klish WJ, Taylor GA *et al.* Relation of body fat indexes to vitamin D status and deficiency among obese adolescents. *Am J Clin Nutr* 2009;90:459-67.

Leslie WD. Ethnic differences in bone mass – clinical implications. *J Clin Endocrinol Metab* 2012;97:4329-40.

Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S, Kusek JW, Van Lente F, Collaboration CKDE. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med* 2006;145:247-54.

Li GW, Chang SX, Xu Z, Chen Y, Bao H, Shi X. Prediction of hip osteoporotic fractures from composite indices of femoral neck strength. *Skeletal Radiol* 2013;42:195-201.

Lin E, Armstrong-Moore D, Liang Z, Sweeney JF, Torres WE, Ziegler TR, Tangpricha V, Gletsu-Miller N. Contribution of adipose tissue to plasma 25-hydroxyvitamin D concentrations during weight loss following gastric bypass surgery. *Obesity (Silver Spring)* 2011;19:588-94.



Lippuner K, Golder M, Greiner R. Epidemiology and direct medical costs of osteoporotic fractures in men and women in Switzerland. *Osteoporos Int* 2005;16 Suppl 2:S8-S17.

Lips P. Worldwide status of vitamin D nutrition. *J Steroid Biochem Mol Biol* 2010;121:297-300.

Liu AY, Vittinghoff E, Sellmeyer DE, Irvin R, Mulligan K, Mayer K, Thompson M, Grant R, Pathak S, O'Hara B, Gvetadze R, Chillag K *et al.* Bone mineral density in HIV-negative men participating in a tenofovir pre-exposure prophylaxis randomized clinical trial in San Francisco. *PLoS One* 2011;6:e23688.

Liu PT, Stenger S, Li H, Wenzel L, Tan BH, Krutzik SR, Ochoa MT, Schauber J, Wu K, Meinken C, Kamen DL, Wagner M *et al.* Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. *Science* 2006;311:1770-3.

Lo Re 3rd V, Guaraldi G, Leonard MB, Localio AR, Lin J, Orlando G, Zirilli L, Rochira V, Kostman JR, Tebas P. Viral hepatitis is associated with reduced bone mineral density in HIV-infected women but not men. *AIDS* 2009;23:2191-8.

Logie D. AIDS cuts life expectancy in sub-saharan Africa by a quarter. *BMJ* 1999;319:806.

Lucas RM, McMichael AJ, Armstrong BK, Smith WT. Estimating the global disease burden due to ultraviolet radiation exposure. *Int J Epidemiol* 2008;37:654-67.

Maagaard A, Holberg-Petersen M, Kollberg G, Oldfors A, Sandvik L, Bruun JN. Mitochondrial (mt)DNA changes in tissue may not be reflected by depletion of mtDNA in peripheral blood mononuclear cells in HIV-infected patients. *Antivir Ther* 2006;11:601-8.

MacKeown JM, Pedro TM, Norris SA. Energy, macro- and micronutrient intake among a true longitudinal group of South African adolescents at two interceptions (2000 and 2003): the Birth-to-Twenty (Bt20) Study. *Public Health Nutr* 2007;10:635-43.

MacLaughlin JA, Anderson RR, Holick MF. Spectral character of sunlight modulates photosynthesis of previtamin D3 and its photoisomers in human skin. *Science* 1982;216:1001-3.

MacPherson P, Moshabela M, Martinson N, Pronyk P. Mortality and loss to follow-up among HAART initiators in rural South Africa. *Trans R Soc Trop Med Hyg* 2009;103:588-93.

Madeddu G, Spanu A, Solinas P, Calia GM, Lovigu C, Chessa F, Mannazzu M, Falchi A, Mura MS, Madeddu G. Bone mass loss and vitamin D metabolism impairment in HIV patients receiving highly active antiretroviral therapy. *Q J Nucl Med Mol Imaging* 2004;48:39-48.

Madeddu G, Spanu A, Chessa F, Calia GM, Lovigu C, Mannazzu M, Falchi A, Sanna D, Mura MS. Serum leptin and bone metabolism in HIV patients treated with highly active antiretroviral therapy. *Q J Nucl Med Mol Imaging* 2009;53:290-301.

Maggi P, Bartolozzi D, Bonfanti P, Calza L, Cherubini C, Di Biagio A, Marcotullio S, Montella F, Montinaro V, Mussini C, Narciso P, Rusconi S *et al.* Renal complications in HIV disease: between present and future. *AIDS Rev* 2012;14:37-53.

Maharaj SS, Chetty V. Rehabilitation program for the quality of life for individuals on highly active antiretroviral therapy in KwaZulu-Natal, South Africa: a short report. *Int J Rehabil Res* 2011;34:360-5.

Malabanan A, Veronikis IE, Holick MF. Redefining vitamin D insufficiency. *Lancet* 1998;351:805-6.

Mallal SA, John M, Moore CB, James IR, McKinnon EJ. Contribution of nucleoside analogue reverse transcriptase inhibitors to subcutaneous fat wasting in patients with HIV infection. *AIDS* 2000;14:1309-16.

Mallon PW, Miller J, Cooper DA, Carr A. Prospective evaluation of the effects of antiretroviral therapy on body composition in HIV-1-infected men starting therapy. *AIDS* 2003;17:971-9.

Mallon PW. HIV and bone mineral density. *Curr Opin Infect Dis* 2010;23:1-8.

Mann J. AIDS: A worldwide pandemic. In: Gottlieb MS, Jeffries DJ, Mildvan D, Pinching, AJ, Quinn TC. Hoboken, eds. *Current topics in AIDS, volume 2*: Chichester: John Wiley & Sons, 1989.

Marquez PV, Farrington JL. No more disease silos for sub-Saharan Africa. *BMJ* 2012;345:e5812.

Marshall D, Johnell O, Wedel H. Meta-analysis of how well measures of bone mineral density predict occurrence of osteoporotic fractures. *BMJ* 1996;312:1254-9.

Martin A, Liu S, David V, Li H, Karydis A, Feng JQ, Quarles LD. Bone proteins PHEX and DMP1 regulate fibroblastic growth factor Fgf23 expression in osteocytes through a common pathway involving FGF receptor (FGFR) signaling. *FASEB J* 2011;25:2551-62.

Martineau AR, Honecker FU, Wilkinson RJ, Griffiths CJ. Vitamin D in the treatment of pulmonary tuberculosis. *J Steroid Biochem Mol Biol* 2007a;103:793-8.

Martineau AR, Wilkinson RJ, Wilkinson KA, Newton SM, Kampmann B, Hall BM, Packe GE, Davidson RN, Eldridge SM, Maunsell ZJ, Rainbow SJ, Berry JL *et al.* A single dose of vitamin D enhances immunity to mycobacteria. *Am J Respir Crit Care Med* 2007b;176:208-13.

Martineau AR, Timms PM, Bothamley GH, Hanifa Y, Islam K, Claxton AP, Packe GE, Moore-Gillon JC, Darmalingam M, Davidson RN, Milburn HJ, Baker LV *et al.* High-dose vitamin D(3) during intensive-phase antimicrobial treatment of pulmonary tuberculosis: a double-blind randomised controlled trial. *Lancet* 2011;377:242-50.

Masiá M, Padilla S, Robledano C, López N, Ramos JM, Gutiérrez F. Early changes in parathyroid hormone concentrations in HIV-infected patients initiating antiretroviral therapy with tenofovir. *AIDS Res Hum Retroviruses* 2012;28:242-6.

Maskew M, MacPhail P, Menezes C, Rubel D. Lost to follow up: contributing factors and challenges in South African patients on antiretroviral therapy. *S Afr Med J* 2007;97:853-7.

Mathieu C, Adorini L. The coming of age of 1,25-dihydroxyvitamin D(3) analogs as immunomodulatory agents. *Trends Mol Med* 2002;8:174-9.



May A, Pettifor JM, Norris SA, Ramsay M, Lombard Z. Genetic factors influencing bone mineral content in a black South African population. *J Bone Miner Metab* 2013;epub ahead of print.

Mayo Clinic. Test ID: RTRPP - Tubular Reabsorption of Phosphorus, Random. http://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/88546. (accessed Oct 2012).

Mayosi BM, Flisher AJ, Lalloo UG, Sitas F, Tollman SM, Bradshaw D. The burden of non-communicable diseases in South Africa. *Lancet* 2009;374:934-47.

Mazess RB, Barden HS, Bisek JP, Hanson J. Dual-energy x-ray absorptiometry for total-body and regional bone-mineral and soft-tissue composition. *Am J Clin Nutr* 1990;51:1106-12.

Mazess RB, Barden HS, Eberle RW, Denton MD. Age changes of spine density in posterior-anterior and lateral projections in normal women. *Calcif Tissue Int* 1995;56:201-5.

McComsey GA, Kendall MA, Tebas P, Swindells S, Hogg E, Alston-Smith B, Suckow C, Gopalakrishnan G, Benson C, Wohl DA. Alendronate with calcium and vitamin D supplementation is safe and effective for the treatment of decreased bone mineral density in HIV. *AIDS* 2007;21:2473-82.

McComsey GA, Kitch D, Daar ES, Tierney C, Jahed NC, Tebas P, Myers L, Melbourne K, Ha B, Sax PE. Bone mineral density and fractures in antiretroviral-naive persons randomized to receive abacavir-lamivudine or tenofovir disoproxil fumarate-emtricitabine along with efavirenz or atazanavir-ritonavir: Aids Clinical Trials Group A5224s, a substudy of ACTG A5202. *J Infect Dis* 2011;203:1791-801.

Mehta P, Nelson M, Brand A, Boag F. Avascular necrosis in HIV. *Rheumatol Int* 2013; 33:235-8.

Mehta S, Giovannucci E, Mugusi FM, Spiegelman D, Aboud S, Hertzmark E, Msamanga GI, Hunter D, Fawzi WW. Vitamin D status of HIV-infected women and its association with HIV disease progression, anemia, and mortality. *PLoS One* 2010;5:e8770.

Micklesfield LK, Reid S, Bewerunge L, Rush E, Goedecke JH. A proposed method to measure body composition in obese individuals using dual-energy X-ray absorptiometry. *Int J Body Comp Res* 2007;5:147.

Moayyeri A, Kaptoge S, Dalzell N, Bingham S, Luben RN, Wareham NJ, Reeve J, Khaw KT. Is QUS or DXA better for predicting the 10-year absolute risk of fracture? *J Bone Miner Res* 2009;24:1319-25.

Mondy K, Yarasheski K, Powderly WG, Whyte M, Claxton S, DeMarco D, Hoffmann M, Tebas P. Longitudinal evolution of bone mineral density and bone markers in human immunodeficiency virus-infected individuals. *Clin Infect Dis* 2003;36:482-90.

Moore AL, Vashisht A, Sabin CA, Mocroft A, Madge S, Phillips AN, Studd JW, Johnson MA. Reduced bone mineral density in HIV-positive individuals. *AIDS* 2001;15:1731-3.

Motsoaledi P. Republic of South Africa, Country Progress report on the declaration of commitment on HIV/AIDS, 2010 report. In: Department of Health, ed. UNAIDS: UNAIDS, 2010:1-126.

Movsesyan L, Tankó LB, Larsen PJ, Christiansen C, Svendsen OL. Variations in percentage of body fat within different BMI groups in young, middle-aged and old women. *Clin Physiol Funct Imaging* 2003;23:130-3.

MRC (SA) SA. Nutritional Intervention Research Unit (NIRU) and Biomedical Research Division (BIRD). http://foodfinder.mrc.ac.za/. (accessed October 2012).

Mueller NJ, Fux CA, Ledergerber B, Elzi L, Schmid P, Dang T, Magenta L, Calmy A, Vergopoulos A, Bischoff-Ferrari HA. High prevalence of severe vitamin D deficiency in combined antiretroviral therapy-naive and successfully treated Swiss HIV patients. *AIDS* 2010;24:1127-34.

Mulligan K, Harris DR, Emmanuel P, Fielding RA, Worrell C, Kapogiannis BG, Monte D, Sleasman J, Wilson CM, Aldrovandi GM. Low bone mass in behaviorally HIV-infected young men on antiretroviral therapy: Adolescent Trials Network Study 021B. *Clin Infect Dis* 2012;55:461-8.

Mupere E, Malone L, Zalwango S, Chiunda A, Okwera A, Parraga I, Stein CM, Tisch DJ, Mugerwa R, Boom WH, Mayanja H, Whalen CC. Lean tissue mass wasting is associated with increased risk of mortality among women with pulmonary tuberculosis in urban Uganda. *Ann Epidemiol* 2012;22:466-73.

Mutimura E, Anastos K, Zheng Lin, Cohen M, Binagwaho A, Kotler DP. Effect of HIV infection on body composition and fat distribution in Rwandan women. *J Int Assoc Physicians AIDS Care (Chic)* 2010;9:173-8.

Nachega JB, Trotta MP, Nelson M, Ammassari A. Impact of metabolic complications on antiretroviral treatment adherence: clinical and public health implications. *Curr HIV/AIDS Rep* 2009;6:121-9.

Naicker N, Norris SA, Mathee A, Becker P, Richter L. Lead exposure is associated with a delay in the onset of puberty in South African adolescent females: findings from the Birth to Twenty cohort. *Sci Total Environ* 2010;408:4949-54.

Nelson DA, Pettifor JM, Norris SA. Race, Ethnicity, and Osteoporosis. In: Marcus R, Feldman D, eds. *Osteoporosis*. San Diego: Elsevier, 2008:667-89.

Nelson M, Haraldsdóttir J. Food photographs: practical guidelines I. Design and analysis of studies to validate portion size estimates. *Public Health Nutr* 1998a;1:219-30.

Nelson M, Haraldsdóttir J. Food photographs: practical guidelines II. Development and use of photographic atlases for assessing food portion size. *Public Health Nutr* 1998b;1:231-7.

NESCA. Nuclear waste. http://www.necsa.co.za/Necsa/Nuclear-Technology/Nuclear-Waste-442.aspx. (accessed May 2010).

New SA. Nutrition Society Medal lecture. The role of the skeleton in acid-base homeostasis. *Proc Nutr Soc* 2002;61:151-64.

New SA, MacDonald HM, Campbell MK, Martin JC, Garton MJ, Robins SP, Reid DM. Lower estimates of net endogenous non-carbonic acid production are positively associated with indexes of bone health in premenopausal and perimenopausal women. *Am J Clin Nutr* 2004;79:131-8.

NIAID. HIV/AIDS Biology of HIV. http://www.meds.com/hiv/hivindex3.html. (accessed May 2010).



National Institute for Clinical Excellence. NICE Quick reference guide Hip fracture.

Nielsen SP. The fallacy of BMD: a critical review of the diagnostic use of dual X-ray absorptiometry. *Clin Rheumatol* 2000;19:174-83.

Nishijima T, Gatanaga H, Komatsu H, Tsukada K, Shimbo T, Aoki T, Watanabe K, Kinai E, Honda H, Tanuma J, Yazaki H, Honda M *et al.* Renal function declines more in tenofovir- than abacavir-based antiretroviral therapy in low-body weight treatment-naive patients with HIV infection. *PLoS One* 2012;7:e29977.

(NOS) National Osteoporosis Society. Scans and tests and osteoporosis. 2012;NOS/00147.

Ohl J, Partisani M, Demangeat C, Binder-Foucard F, Nisand I, Lang JM. Alterations of ovarian reserve tests in Human Immunodeficiency Virus (HIV)-infected women. *Gynecol Obstet Fertil* 2010;38:313-7.

Olausson H, Goldberg GR, Laskey MA, Schoenmakers I, Jarjou LM, Prentice A. Calcium economy in human pregnancy and lactation. *Nutr Res Rev* 2012;25:40-67.

Olds WJ, McKinley AR, Moore MR, Kimlin MG. In vitro model of vitamin D3 (cholecalciferol) synthesis by UV radiation: dose-response relationships. *J Photochem Photobiol B* 2008;93:88-93.

Ott SM. ACP Journal Club. Review: Vitamin D with calcium reduces fractures in adults. *Ann Intern Med* 2012a;156:JC6-7.

Ott SM. Vitamin d dose requirements for fracture prevention. *N Engl J Med* 2012b;367:1367-70.

Paccou J, Viget N, Legrout-Gerot I, Yazdanpanah Y, Cortet B. Bone loss in patients with HIV infection. *Joint Bone Spine* 2009;76:637-41.

Palella FJ, Jr, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, Aschman DJ, Holmberg SD. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. *N Engl J Med* 1998;338:853-60.

Pan G, Yang Z, Ballinger SW, McDonald JM. Pathogenesis of osteopenia/osteoporosis induced by highly active anti-retroviral therapy for AIDS. *Ann N Y Acad Sci* 2006;1068:297-308.

Parry CD, Carney T, Petersen P, Dewing S, Needle R. HIV-risk behavior among injecting or non-injecting drug users in Cape Town, Pretoria, and Durban, South Africa. *Subst Use Misuse* 2009;44:886-904.

Pascussi JM, Robert A, Nguyen M, Walrant-Debray O, Garabedian M, Martin P, Pineau T, Saric J, Navarro F, Maurel P, Vilarem MJ. Possible involvement of pregnane X receptor-enhanced CYP24 expression in drug-induced osteomalacia. *J Clin Invest* 2005;115:177-86.

Paton NI, Macallan DC, Griffin GE, Pazianas M. Bone mineral density in patients with human immunodeficiency virus infection. *Calcif Tissue Int* 1997;61:30-2.

Payne RB. Renal tubular reabsorption of phosphate (TmP/GFR): indications and interpretation. *Ann Clin Biochem* 1998;35 ( Pt 2):201-6.

Pedro TM, MacKeown JM, Norris SA. Variety and total number of food items recorded by a true longitudinal group of urban black South African children at five interceptions between 1995 and 2003: the Birth-to-Twenty (Bt20) Study. *Public Health Nutr* 2008;11:616-23.

Pepin J. *The origin of AIDS*. Cambridge University Press, 2011.

Pettifor JM. Vitamin D &/or calcium deficiency rickets in infants & children: a global perspective. *Indian J Med Res* 2008;127:245-9.
Pettifor JM, Ross FP, Solomon L. Seasonal variation in serum 25-hydroxycholecalciferol concentrations in elderly South African patients with fractures of femoral neck. *BMJ* 1978;1:826-7.

Pettifor JM, Moodley GP, Hough FS, Koch H, Chen T, Lu Z, Holick MF. The effect of season and latitude on in vitro vitamin D formation by sunlight in South Africa. *S Afr Med J* 1996;86:1270-2.

Pieribone D. The HIV Life Cycle. http://www.thebody.com/content/art14193.html. (accessed May 2010).

Piwowar-Manning E, Fiamma A, Laeyendecker O, Kulich M, Donnell D, Szekeres G, Robins-Morris L, Mullis CE, Vallari A, Hackett J, Mastro TD, Gray G *et al.* HIV surveillance in a large, community-based study: results from the pilot study of Project Accept (HIV Prevention Trials Network 043). *BMC Infect Dis* 2011;11:251.

Pollock E, Klotsas AE, Compston J, Gkrania-Klotsas E. Bone health in HIV infection. *Br Med Bull* 2009;92:123-33.

de Pommerol M, Hessamfar M, Lawson-Ayayi S, Neau D, Geffard S, Farbos S, Uwamaliya B, Vandenhende MA, Pellegrin JL, Blancpain S, Dabis F, Morlat P *et al.* Menopause and HIV infection: age at onset and associated factors, ANRS CO3 Aquitaine cohort. *Int J STD AIDS* 2011;22:67-72.

Porthouse J, Cockayne S, King C, Saxon L, Steele E, Aspray T, Baverstock M, Birks Y, Dumville J, Francis R, Iglesias C, Puffer S *et al.* Randomised controlled trial of calcium and supplementation with cholecalciferol (vitamin D3) for prevention of fractures in primary care. *BMJ* 2005;330:1003.

Post FA, McCloskey EV, Compston JE, Bowman CA, Hay PE, Johnson MA, Mallon PW, Peters BS, Samarawickrama A, Tudor-Williams G. Prevention of bone loss and management of fracture risk in HIV-infected individuals: case studies and recommendations for different patient subgroups. *Future Virology* 2011;6 (6):769-82.

Powderly W, Cohen C, Gallant Jea. Similar incidence of osteopenia and osteoporosis in ART-naive patients treated with tenofovir DF vs. stavudine in combination with lamivudine and efavirenz over 144 weeks [abstract 823]. 903 study group. 12th conference on Retroviruses and Opportunistic Infections. Boston, 2005.

Premaor MO, Compston JE. Testing for secondary causes of osteoporosis. *BMJ* 2010;341:c6959.

Prentice A. Diet, nutrition and the prevention of osteoporosis. *Public Health Nutrition* 2004;7:227-43.

Prentice A. Vitamin D deficiency: a global perspective. *Nutr Rev* 2008;66:S153-64.



Prentice A, Parsons TJ, Cole TJ. Uncritical use of bone mineral density in absorptiometry may lead to size-related artifacts in the identification of bone mineral determinants. *Am J Clin Nutr* 1994;60:837-42.

Prentice A, Goldberg GR, Schoenmakers I. Vitamin D across the lifecycle:physiology and biomarkers. *Am J Clin Nutr* 2008;88:500S-6S.

Prentice A, Schoenmakers I, Jones KS, Jarjou LMA, Goldberg GR. Vitamin D deficiency and its health consequences in Africa. *Clinic Rev Bone Miner Metab* 2009a;7:94-106.

Prentice RL, Shaw PA, Bingham SA, Beresford SA, Caan B, Neuhouser ML, Patterson RE, Stefanick ML, Satterfield S, Thomson CA, Snetselaar L, Thomas A *et al.* Biomarker-calibrated energy and protein consumption and increased cancer risk among postmenopausal women. *Am J Epidemiol* 2009b;169:977-89.

Prior J, Burdge D, Maan E, Milner R, Hankins C, Klein M, Walmsley S. Fragility fractures and bone mineral density in HIV positive women: a case-control population-based study. *Osteoporos Int* 2007;18:1345-53.

Radiological Society of North America Inc. (RSNA). Radiation Exposure in X-ray Examinations. http://www.radiologyinfo.org/en/safety/index.cfm?pg=sfty_xray#part2. (accessed February 2012).

Raisz LG, Bilezikian JP, Martin TJ. Pathophysiology of Osteoporosis. In: Raisz LG, Bilezikian JP, Martin TJ, eds. *Principles of Bone Biology*. USA: Academic Press, 2008:1635-47.

Ramrakha P, Moore K, Sam A. *Oxford handbook of acute medicine,* 3rd edn. Oxford University Press, 2010.

Rao MN, Schambelan M, Tai VW, Abrams DI, Khatami H, Havel PJ, Sakkas G, Mulligan K. Assessing the association between leptin and bone mineral density in HIV-infected men. *AIDS Res Treat* 2012;2012:103072.

Reid IR. Menopause. *Primer on the metabolic bone diseases and disorders of mineral metabolism*. Washington DC: ASBMR, 2008:95-98.

Richman DD, Fischl MA, Grieco MH, Gottlieb MS, Volberding PA, Laskin OL, Leedom JM, Groopman JE, Mildvan D, Hirsch MS. The toxicity of azidothymidine (AZT) in the treatment of patients with AIDS and AIDS-related complex. A double-blind, placebo-controlled trial. *N Engl J Med* 1987;317:192-7.

Richter L, Norris S, Pettifor J, Yach D, Cameron N. Cohort profile: Mandela's children: the 1990 Birth to Twenty study in South Africa. *Int J Epidemiol* 2007;36:504-11.

Rietschel P, Corcoran C, Stanley T, Basgoz N, Klibanski A, Grinspoon S. Prevalence of hypogonadism among men with weight loss related to human immunodeficiency virus infection who were receiving highly active antiretroviral therapy. *Clin Infect Dis* 2000;31:1240-4.

Rikkonen T, Sirola J, Salovaara K, Tuppurainen M, Jurvelin JS, Honkanen R, Kröger H. Muscle strength and body composition are clinical indicators of osteoporosis. *Calcif Tissue Int* 2012;91:131-8.

Rodriguez M, Daniels B, Gunawardene S, Robbins GK. High frequency of vitamin D deficiency in ambulatory HIV-positive patients. *AIDS Res Hum Retroviruses* 2009;25:9-14.

Rosenfeldt FL, Mijch A, McCrystal G, Sweeney C, Pepe S, Nicholls M, Dennett X. Skeletal myopathy associated with nucleoside reverse transcriptase inhibitor therapy: potential benefit of coenzyme Q10 therapy. *Int J STD AIDS* 2005;16:827-9.

Rosenvigne M, Gedela K, Copas A, Wilkinson A, Sheehy C, Bano G, Hay P, Pakianathan M, Sadiq S. Tenofovir-linked hyperparathyroidism is independently associated with the presence of vitamin D deficiency. *JAIDS* 2010; 54:496-9.

Ross AC. Dietary reference intakes for calcium and vitamin D. IOM, 2010.
Ross PD, Norimatsu H, Davis JW, Yano K, Wasnich RD, Fujiwara S, Hosoda Y, Melton LJ. A comparison of hip fracture incidence among native Japanese, Japanese Americans, and American Caucasians. *Am J Epidemiol* 1991;133:801-9.

Rush EC, Goedecke JH, Jennings C, Micklesfield L, Dugas L, Lambert EV, Plank LD. BMI, fat and muscle differences in urban women of five ethnicities from two countries. *Int J Obes (Lond)* 2007;31:1232-9.

Sage E, Girard PM, Francesconi S. Unravelling UVA-induced mutagenesis. *Photochem Photobiol Sci* 2012;11:74-80.

Sapir-Koren R, Livshits G. Bone mineralization and regulation of phosphate homeostasis. IBMS BoneKey 2011;8:286-300. http://www.nature.com/bonekey/knowledgeenvironment/2011/1106/bonekey20110516/full/bonekey20110516.html.(accessed September 2012).

Schambelan M, Benson CA, Carr A, Currier JS, Dube MP, Gerber JG, Grinspoon SK, Grunfeld C, Kotler DP, Mulligan K, Powderly WG, Saag MS. Management of metabolic complications associated with antiretroviral therapy for HIV-1 infection: recommendations of an International AIDS Society-USA panel. *J Acquir Immune Defic Syndr* 2002;31:257-75.

Schoenmakers I, Goldberg GR, Prentice A. Abundant sunshine and vitamin D deficiency. *Br J Nutr* 2008;99:1171-3.

van Schoor NM, Lips P. Worldwide vitamin D status. *Best Pract Res Clin Endocrinol Metab* 2011;25:671-80.

Scientific Advisory Committee on Nutrition. Update on vitamin D. Norwich: TSO (The Stationery Office), 2007.

Seeman E. Modeling and remodeling. In: Bilezikian JP, Raisz LG, Martin TJ, eds. *Principles of Bone Biology*. USA: Academic Press, 2008:3-29.

Segatto AF, Freitas IF, Santos VR, Alves KC, Barbosa DA, Portelinha AM, Monteiro HL. Indices of body fat distribution for assessment of lipodysthrophy in people living with HIV/AIDS. *BMC Res Notes* 2012;5:2101791285670487.

Seibel MJ. Biochemical markers of bone turnover: part I: biochemistry and variability. *Clin Biochem Rev* 2005;26:97-122.

Seminari E, Castagna A, Soldarini A, Galli L, Fusetti G, Dorigatti F, Hasson H, Danise A, Guffanti M, Lazzarin A, Rubinacci A. Osteoprotegerin and bone turnover markers in heavily pretreated HIV-infected patients. *HIV Med* 2005;6:145-50.

Shahmanesh M, Cartledge J, Miller R. Lactic acidosis and abnormal liver function in advanced HIV disease. *Sex Transm Infect* 2002;78:139-42.



Shepherd JA, Wang L, Fan B, Gilsanz V, Kalkwarf HJ, Lappe J, Lu Y, Hangartner T, Zemel BS, Fredrick M, Oberfield S, Winer KK. Optimal monitoring time interval between DXA measures in children. *J Bone Miner Res* 2011;26:2745-52.

Sheppard ZA, Norris SA, Pettifor JM, Cameron N, Griffiths PL. Approaches for assessing the role of household socioeconomic status on child anthropometric measures in urban South Africa. *Am J Hum Biol* 2009;21:48-54.

Siervo M, Frühbeck G, Dixon A, Goldberg GR, Coward WA, Murgatroyd PR, Prentice AM, Jebb SA. Efficiency of autoregulatory homeostatic responses to imposed caloric excess in lean men. *Am J Physiol Endocrinol Metab* 2008;294:E416-24.

Sigve. HIV-timecourse. http://en.wikipedia.org/w/index.php?title=File:Hiv-timecourse_copy.svg&page=1. (accessed Jan 2013).

de Silva TI, Cotten M, Rowland-Jones SL. HIV-2: the forgotten AIDS virus. *Trends Microbiol* 2008;16:588-95.

de Silva TI, van Tienen C, Onyango C, Jabang A, Vincent T, Loeff MF, Coutinho RA, Jaye A, Rowland-Jones S, Whittle H, Cotten M, Hué S. Population dynamics of HIV-2 in rural Guinea-Bissau: comparison with HIV-1 and ongoing transmission at the heart of the epidemic. *AIDS* 2012;27:125-34.

Sliwa K, Carrington MJ, Becker A, Thienemann F, Ntsekhe M, Stewart S. Contribution of the human immunodeficiency virus/acquired immunodeficiency syndrome epidemic to de novo presentations of heart disease in the Heart of Soweto Study cohort. *Eur Heart J* 2012;33:866-74.

De Socio GV, Fabbriciani G, Massarotti M, Messina S, Cecchini E, Marasini B. Hypophosphatemic osteomalacia associated with tenofovir: a multidisciplinary approach is required. *Mediterr J Hematol Infect Dis* 2012;4:e2012025.

Smith AW, Hendrickse RG, Harrison C, Hayes RJ, Greenwood BM. The effects on malaria of treatment of iron-deficiency anaemia with oral iron in Gambian children. *Ann Trop Paediatr* 1989;9:17-23.

Health Physics Society. Radiation exposure from medical diagnostic imaging procedures. http://hps.org/documents/meddiagimaging.pdf. (accessed August 2012).

South African Department of Health: Motsoaledi. Clinical guidelines for the management of HIV and AIDS in adults and adolescents. In: National Department of Health, ed., 2010.

Souverein OW, de Boer WJ, Geelen A, van der Voet H, de Vries JH, Feinberg M, van't Veer P. Uncertainty in intake due to portion size estimation in 24-hour recalls varies between food groups. *J Nutr* 2011;141:1396-401.

Stear S. The influence of diet and exercise on bone mineral status during adolescence. PhD thesis 1998. University of Cambridge.

Stein EM, Yin MT, McMahon DJ, Shu A, Zhang CA, Ferris DC, Colon I, Dobkin JF, Hammer SM, Shane E. Vitamin D deficiency in HIV-infected postmenopausal Hispanic and African-American women. *Osteoporos Int* 2011;22:477-87.

Stellbrink HJ, Orkin C, Arribas JR, Compston J, Gerstoft J, Van Wijngaerden E *et al*. Comparison of changes in bone density and turnover with abacavir-lamivudine versus

tenofovir-emtricitabine in HIV-infected adults: 48-week results from the ASSERT study. *Clin Infect Dis*. 2010;51:963-72.

Stevens LA, Schmid CH, Greene T, Zhang YL, Beck GJ, Froissart M, Hamm LL, Lewis JB, Mauer M, Navis GJ, Steffes MW, Eggers PW *et al.* Comparative performance of the CKD Epidemiology Collaboration (CKD-EPI) and the Modification of Diet in Renal Disease (MDRD) Study equations for estimating GFR levels above 60 mL/min/1.73 $m^2$. *Am J Kidney Dis* 2010;56:486-95.

Steyn NP, Nel JH, Casey A. Secondary data analyses of dietary surveys undertaken in South Africa to determine usual food consumption of the population. *Public Health Nutr* 2003;6:631-44.

Steyn NP, Senekal M, Norris SA, Whati L, Mackeown JM, Nel JH. How well do adolescents determine portion sizes of foods and beverages? *Asia Pac J Clin Nutr* 2006;15:35-42.

Stoffels K, Overbergh L, Giulietti A, Verlinden L, Bouillon R, Mathieu C. Immune regulation of 25-hydroxyvitamin-D3-1alpha-hydroxylase in human monocytes. *J Bone Miner Res* 2006;21:37-47.

Stone B, Dockrell D, Bowman C, McCloskey E. HIV and bone disease. *Arch Biochem Biophys* 2010;503:66-77.

Sudfeld CR, Wang M, Aboud S, Giovannucci EL, Mugusi FM, Fawzi WW. Vitamin D and HIV progression among Tanzanian adults initiating antiretroviral therapy. *PLoS One* 2012;7:e40036.

Sudfeld CR, Isanaka S, Aboud S, Mugusi FM, Wang M, Chalamilla GE, Fawzi WW. Association of serum albumin concentration with mortality, morbidity, CD4 T-cell reconstitution among Tanzanians initiating antiretroviral therapy. *J Infect Dis* 2013;207:1370-8.

Sulistyoningrum DC, Green TJ, Lear SA, Devlin AM. Ethnic-specific differences in vitamin D status is associated with adiposity. *PLoS One* 2012;7:e43159.

Svendsen OL, Haarbo J, Hassager C, Christiansen C. Accuracy of measurements of body composition by dual-energy x-ray absorptiometry in vivo. *Am J Clin Nutr* 1993;57:605-8.

Tang AM, Graham NM, Saah AJ. Effects of micronutrient intake on survival in human immunodeficiency virus type 1 infection. *Am J Epidemiol* 1996;143:1244-56.

Tebas P, Powderly WG, Claxton S, Marin D, Tantisiriwat W, Teitelbaum SL, Yarasheski KE. Accelerated bone mineral loss in HIV-infected patients receiving potent antiretroviral therapy. *AIDS* 2000;14:F63-7.

Terzian AS, Holman S, Nathwani N, Robison E, Weber K, Young M, Greenblatt RM, Gange SJ. Factors associated with preclinical disability and frailty among HIV-infected and HIV-uninfected women in the era of cART. *J Womens Health (Larchmt)* 2009;18:1965-74.

The Antiretroviral Therapy Cohort Collaboration. Life expectancy of individuals on combination antiretroviral therapy in high-income countries: a collaborative analysis of 14 cohort studies. *Lancet* 2008;372:293–9.

Torti C, Mazziotti G, Soldini PA, Focà E, Maroldi R, Gotti D, Carosi G, Giustina A. High prevalence of radiological vertebral fractures in HIV-infected males. *Endocrine* 2012;41:512-17.



Tothill P. Dual-energy X-ray absorptiometry for the measurement of bone and soft tissue composition. *Clin Nutr* 1995;14:263-8.

Tothill P, Avenell A. Anomalies in the measurement of changes in bone mineral density of the spine by dual-energy X-ray absorptiometry. *Calcif Tissue Int* 1998;63:126-33.

Tothill P, Avenell A, Reid DM. Precision and accuracy of measurements of whole-body bone mineral: comparisons between Hologic, Lunar and Norland dual-energy X-ray absorptiometers. *Br J Radiol* 1994;67:1210-17.

Tothill P, Hannan WJ, Cowen S, Freeman CP. Anomalies in the measurement of changes in total-body bone mineral by dual-energy X-ray absorptiometry during weight change. *J Bone Miner Res* 1997;12:1908-21.

Triant VA, Brown TT, Lee H, Grinspoon SK. Fracture prevalence among human immunodeficiency virus (HIV)-infected versus non-HIV-infected patients in a large US healthcare system. *J Clin Endocrinol Metab* 2008;93:3499-504.

Trumbo P, Schlicker S, Yates AA, Poos M. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. *J Am Diet Assoc* 2002;102:1621-30.

Turner RT, Sibonga JD. Effects of alcohol use and estrogen on bone. *Alcohol Res Health* 2001;25:276-81.

UNAIDS. Report on the global AIDS epidemic. UNAIDS, 2008a:1-36.

UNAIDS. Towards universal access scaling up priority HIV/AIDS interventions in the health sector. Progress report 2008. http://www.who.int/hiv/pub/towards_universal_access_report_2008.pdf. (accessed May 2012)

UNAIDS. World overview. http://www.unaids.org/en/dataanalysis/datatools/aidsinfo/. (accessed October 2012).

University of Birmingham at Alabama. Hormonal control of calcium homeostasis. http://students.cis.uab.edu/elo/finalproject.html. (accessed September 2012).

UNSCEAR. Report of the United Nations Scientific Committee on the Effects of Atomic Radiation to the General Assembly. http://www.unscear.org/docs/reports/gareport.pdf. (accessed May 2010).

Valizadeh M, Mazloomzadeh S, Golmohammadi S, Larijani B. Mortality after low trauma hip fracture: a prospective cohort study. *BMC Musculoskelet Disord* 2012;13:143.

Van Den Bout-Van Den Beukel CJ, Fievez L, Michels M, Sweep FC, Hermus AR, Bosch ME, Burger DM, Bravenboer B, Koopmans PP, Van Der Ven AJ. Vitamin D deficiency among HIV type 1-infected individuals in the Netherlands: effects of antiretroviral therapy. *AIDS Res Hum Retroviruses* 2008a;24:1375-82.

Van den Bout-van den Beukel CJ, van den Bos M, Oyen WJ, Hermus AR, Sweep FC, Tack CJ, Bosch ME, Burger DM, Koopmans PP, van der Ven AJ. The effect of cholecalciferol supplementation on vitamin D levels and insulin sensitivity is dose related in vitamin D-deficient HIV-1-infected patients. *HIV Med* 2008b;9:771-9.

Viard JP, Souberbielle JC, Kirk O, Reekie J, Knysz B, Losso M, Gatell J, Pedersen C, Bogner JR, Lundgren JD, Mocroft A. Vitamin D and clinical disease progression in HIV infection: results from the EuroSIDA study. *AIDS* 2011;25:1305-15.

Villamor E. A potential role for vitamin D on HIV infection? *Nutr Rev* 2006;64:226-33.

Villamor E, Koulinska IN, Aboud S, Murrin C, Bosch RJ, Manji KP, Fawzi WW. Effect of vitamin supplements on HIV shedding in breast milk. *Am J Clin Nutr* 2010;92:881-6.

Wainwright SA, Marshall LM, Ensrud KE, Cauley JA, Black DM, Hillier TA, Hochberg MC, Vogt MT, Orwoll ES, Group SoOFR. Hip fracture in women without osteoporosis. *J Clin Endocrinol Metab* 2005;90:2787-93.

Walker-Bone K. HIV infection and the risk of secondary osteoporosis. Osteoporosis Review 2010 Spring 2010:1-6.
Walker-Bone K. Recognizing and treating secondary osteoporosis. *Nat Rev Rheumatol* 2012;8:480-92.

Wang S. Epidemiology of vitamin D in health and disease. *Nutr Res Rev* 2009;22:188-203.

Ward K. Musculoskeletal phenotype through the life course: the role of nutrition. *Proc Nutr Soc* 2012;71:27-37.

Webb R. The influence of adipose tissue on bone health. PhD thesis 2008. University of Cambridge.

Wejse C, Olesen R, Rabna P, Kaestel P, Gustafson P, Aaby P, Andersen PL, Glerup H, Sodemann M. Serum 25-hydroxyvitamin D in a West African population of tuberculosis patients and unmatched healthy controls. *Am J Clin Nutr* 2007;86:1376-83.

Welz T, Childs K, Ibrahim F, Poulton M, Taylor CB, Moniz CF, Post FA. Efavirenz is associated with severe vitamin D deficiency and increased alkaline phosphatase. *AIDS* 2010;24:1923-8.

WHO. Recommendations for preventing osteoporosis. www.who.int/entity/dietphysicalactivity/publications/trs916/en/gsfao_osteo.pdf. (accessed June 2012).

WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet* 2004;363:157-63.

WHO. WHO Technical Report 2003 "Prevention and management of osteoporosis". 2003.
WHO. BMI classification. http://apps.who.int/bmi/index.jsp?introPage=intro_3.html. (accessed January 2013).

Wikvall K. Cytochrome P450 enzymes in the bioactivation of vitamin D to its hormonal form (review). *Int J Mol Med* 2001;7:201-9.

Wilkinson RJ, Llewelyn M, Toossi Z, Patel P, Pasvol G, Lalvani A, Wright D, Latif M, Davidson RN. Influence of vitamin D deficiency and vitamin D receptor polymorphisms on tuberculosis among Gujarati Asians in west London: a case-control study. *Lancet* 2000;355:618-21.

Willey BA, Cameron N, Norris SA, Pettifor JM, Griffiths PL. Socio-economic predictors of stunting in preschool children – a population-based study from Johannesburg and Soweto. *S Afr Med J* 2009;99:450-6.



Wolmarans P, Danster N, Dalton A, Rossouw K, Schönfeldt H. Condensed food composition tables for South Africa (2010). In: Medical Research Council, eds. Cape Town, 2010.

Woodward CL, Hall AM, Williams IG, Madge S, Copas A, Nair D, Edwards SG, Johnson MA, Connolly JO. Tenofovir-associated renal and bone toxicity. *HIV Med* 2009;10:482-7.

Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr* 2000;72:690-3.

Wrottesley S. The impact of HIV on dietary intake and body composition: a comparison between HIV-positive and HIV-negative urban South African women. MSc Thesis 2011. London School of Hygiene and Tropical Medicine.
Wrottesley S, Micklesfield L, Hamill M, Goldberg G, Prentice A, Pettifor J, Norris S, Feeley A. Dietary intake and body composition in HIV-positive and -negative South African women *Public Health Nutrition* 2013;In press.

Wyatt CM, Meliambro K, Klotman PE. Recent progress in HIV-associated nephropathy. *Annu Rev Med* 2012;63:147-59.

Xu Y, Hashizume T, Shuhart MC, Davis CL, Nelson WL, Sakaki T, Kalhorn TF, Watkins PB, Schuetz EG, Thummel KE. Intestinal and hepatic CYP3A4 catalyze hydroxylation of 1alpha,25-dihydroxyvitamin D(3): implications for drug-induced osteomalacia. *Mol Pharmacol* 2006;69:56-65.

Yan L, Zhou B, Wang X, D'Ath S, Laidlaw A, Laskey MA, Prentice A. Older people in China and the United Kingdom differ in the relationships among parathyroid hormone, vitamin D, and bone mineral status. *Bone* 2003;33:620-7.

Yin MT, McMahon DJ, Ferris DC, Zhang CA, Shu A, Staron R, Colon I, Laurence J, Dobkin JF, Hammer SM, Shane E. Low bone mass and high bone turnover in postmenopausal human immunodeficiency virus-infected women. *J Clin Endocrinol Metab* 2009; doi: 10.1210/jc.2009-0708.

Yong MK, Elliott JH, Woolley IJ, Hoy JF. Low CD4 count is associated with an increased risk of fragility fracture in HIV-infected patients. *J Acquir Immune Defic Syndr* 2011; 1;57:205-10.

Yoon HK, Park C, Jang S, Lee YK, Ha YC. Incidence and mortality following hip fracture in Korea. *J Korean Med Sci* 2011;26:1087-92.

Yu EW, Thomas BJ, Brown JK, Finkelstein JS. Simulated increases in body fat and errors in bone mineral density measurements by DXA and QCT. *J Bone Miner Res* 2011;DOI: 10.1002/jbmr.506.

Zebaze RM, Seeman E. Epidemiology of hip and wrist fractures in Cameroon, Africa. *Osteoporos Int* 2003;14:301-5.

Zingoni C. To what extent have urban South African adolescents experienced the nutrition transition? PhD thesis 2007. Loughborough University.

Zingoni C, Norris SA, Griffiths PL, Cameron N. Studying a population undergoing nutrition transition: a practical case study of dietary assessment in urban South African adolescents. *Ecol Food Nutr* 2009;48:178-98.

## Appendix 1 Full list of ART

(http://www.fda.gov/ForConsumers/byAudience/ForPatientAdvocates/HIVandAIDSActivities/ucm118915.htm) (Accessed 30/2/2013)

**Non Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)**

| Brand Name | Generic Name | Manufacturer Name* | Approval Date | Time to Approval |
|---|---|---|---|---|
| Atripla | efavirenz, emtricitabine and tenofovir disoproxil fumarate | Bristol-Myers Squibb and Gilead Sciences | 12-July-06 | 2.5 months |
| Complera | emtricitabine, rilpivirine, and tenofovir disoproxil fumarate | Gilead Sciences | 10-August-11 | 6 months |
| Stribild | elvitegravir, cobicistat, emtricitabine, tenofovir disoproxil fumarate | Gilead Sciences | 27-August-12 | 6 months |

Nucleoside Reverse Transcriptase Inhibitors (NRTIs)

| Brand Name | Generic Name | Manufacturer Name* | Approval Date | Time to Approval |
|---|---|---|---|---|
| Combivir | lamivudine and zidovudine | GlaxoSmithKline | 27-Sep-97 | 3.9 months |
| Emtriva | emtricitabine, FTC | Gilead Sciences | 02-Jul-03 | 10 months |
| Epivir | lamivudine, 3TC | GlaxoSmithKline | 17-Nov-95 | 4.4 months |
| Epzicom | abacavir and lamivudine | GlaxoSmithKline | 02-Aug-04 | 10 months |
| Hivid | zalcitabine, dideoxycytidine, ddC (no longer marketed) | Hoffmann-La Roche | 19-Jun-92 | 7.6 months |
| Retrovir | zidovudine, azidothymidine, AZT, ZDV | GlaxoSmithKline | 19-Mar-87 | 3.5 months |
| Trizivir | abacavir, zidovudine, and lamivudine | GlaxoSmithKline | 14-Nov-00 | 10.9 months |
| Truvada | tenofovir disoproxil fumarate and emtricitabine | Gilead Sciences, Inc. | 02-Aug-04 | 5 months |
| Videx EC | enteric coated didanosine, ddI EC | Bristol Myers-Squibb | 31-Oct-00 | 9 months |
| Videx | didanosine, dideoxyinosine, ddI | Bristol Myers-Squibb | 9-Oct-91 | 6 months |
| Viread | tenofovir disoproxil | Gilead | 26-Oct-01 | 5.9 months |

| | | | | |
|---|---|---|---|---|
| | fumarate, TDF | | | |
| Zerit | stavudine, d4T | Bristol Myers-Squibb | 24-Jun-94 | 5.9 months |
| Ziagen | abacavir sulfate, ABC | GlaxoSmithKline | 17-Dec-98 | 5.8 months |

Non Nucleoside Reverse Transcriptase Inhibitors (NNRTIs)

| Brand Name | Generic Name | Manufacturer Name* | Approval Date | Time to Approval |
|---|---|---|---|---|
| Edurant | rilpivirine | Tibotec Therapeutics | 20-May-11 | 10 months |
| Intelence | etravirine | Tibotec Therapeutics | 18-Jan-08 | 6 months |
| Rescriptor | delavirdine, DLV | Pfizer | 4-Apr-97 | 8.7 months |
| Sustiva | efavirenz, EFV | Bristol Myers-Squibb | 17-Sep-98 | 3.2 months |
| Viramune (Immediate Release) | nevirapine, NVP | Boehringer Ingelheim | 21-Jun-96 | 3.9 months |
| Viramune XR(Extended Release) | nevirapine, NVP | Boehringer Ingelheim | 25-Mar-11 | 9.9 months |

Protease Inhibitors (PIs)

| Brand Name | Generic Name | Manufacturer Name* | Approval Date | Time to Approval |
|---|---|---|---|---|
| Agenerase | amprenavir, APV (no longer marketed) | GlaxoSmithKline | 15-Apr-99 | 6 months |
| Aptivus | tipranavir, TPV | Boehringer Ingelheim | 22-Jun-05 | 6 months |
| Crixivan | indinavir, IDV, | Merck | 13-Mar-96 | 1.4 months |
| Fortovase | saquinavir (no longer marketed) | Hoffmann-La Roche | 7-Nov-97 | 5.9 months |
| Invirase | saquinavir mesylate, SQV | Hoffmann-La Roche | 6-Dec-95 | 3.2 months |
| Kaletra | lopinavir and ritonavir, LPV/RTV | Abbott Laboratories | 15-Sep-00 | 3.5 months |
| Lexiva | Fosamprenavir Calcium, FOS-APV | GlaxoSmithKline | 20-Oct-03 | 10 months |
| Norvir | ritonavir, RTV | Abbott Laboratories | 1-Mar-96 | 2.3 months |
| Prezista | darunavir | Tibotec, Inc. | 23-Jun-06 | 6 months |
| Reyataz | atazanavir sulfate, ATV | Bristol-Myers Squibb | 20-Jun-03 | 6 months |
| Viracept | nelfinavir mesylate, NFV | Agouron Pharmaceuticals | 14-Mar-97 | 2.6 months |

Fusion Inhibitors

| Brand | Generic Name | Manufacturer Name | Approval | Time to |
|---|---|---|---|---|



| Name | | | Date | Approval |
|---|---|---|---|---|
| Fuzeon | enfuvirtide, T-20 | Hoffmann-La Roche & Trimeris | 13-Mar-03 | 6 months |

Entry Inhibitors - CCR5 co-receptor antagonist

| Brand Name | Generic Name | Manufacturer Name | Approval Date | Time to Approval |
|---|---|---|---|---|
| Selzentry | Maraviroc | Pfizer | 06-August-07 | 8 months |

HIV integrase strand transfer inhibitors

| Brand Name | Generic Name | Manufacturer Name | Approval Date | Time to Approval |
|---|---|---|---|---|
| Isentress | Raltegravir | Merck & Co., Inc. | 12--Oct-07 | 6 months |

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**WOMEN'S BONE STUDY (WBS)**
Characteristics and health data collection follow up

WBS number: | | | | | | | Month of follow up visit (circle):

| 6 | 12 | 24 | 36 |
|---|---|---|---|

Date: _____/_____/_____

| Group | **Group 1** | | **Group 2** | | **Group 3** | |
|---|---|---|---|---|---|---|
| 1. Repeat HIV test in past 6 weeks<br><br>Result (0=negative, 1=positive) | | Y☐ N☐<br><br>0☐ 1☐ | | x | | x |
| 2. Pregnancy test Negative<br><br>(if positive inform Dr Hamill) | | Y☐ N☐ | | Y☐ N☐ | | Y☐ N☐ |



| | | | | | | |
|---|---|---|---|---|---|---|
| 3. Pregnancy since last visit? (if Yes inform Dr Hamill) | | Y☐ N☐ | | Y☐ N☐ | | Y☐ N☐ |
| - If currently pregnant: Estimated duration (gestation) in weeks (inform Dr Hamill) | | | | | | |
| 4. Currently breastfeeding (if Yes inform Dr Hamill) | | Y☐ N☐ | | Y☐ N☐ | | Y☐ N☐ |
| 5. Regular periods | | Y☐ N☐ | | Y☐ N☐ | | Y☐ N☐ |
| 6. Reached menopause (if Yes inform Dr Hamill)<br><br>- If Y, when was last period (age) _____Yrs_____Mnths<br><br>- Notes: | | Y☐ N☐ | | Y☐ N☐ | | Y☐ N☐ |
| 7. Using hormonal contraception<br><br>- If Y, what (e.g. the pill, depo provera): | | Y☐ N☐ | | Y☐ N☐ | | Y☐ N☐ |
| 8. Sterilisation | | Y☐ N☐ | | Y☐ N☐ | | Y☐ N☐ |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 9. Other family planning e.g. condoms<br><br>List here: | | Y☐ N☐ | | Y☐ N☐ | | Y☐ N☐ | | |
| 10. Age in years & months e.g. 37Yrs 6 Mnths | | | | | | | | |
| 11. Current smoker | | Y☐ N☐ | | Y☐ N☐ | | Y☐ N☐ | | |
| 12. Started smoking after last visit? | | Y☐ N☐ | | Y☐ N☐ | | Y☐ N☐ | | |
| 13. Total month/years of smoking | | | | | | | | |
| 14. How many cigarette do you smoke per day | | | | | | | | |
| 15. **New** fracture since last visit (if 'No' go to Q. 19) | | Y☐ N☐ | | Y☐ N☐ | | Y☐ N☐ | | |
| 16. Site of this fracture (e.g. left hip): | | | | | | | | |
| 17. Was it a **traumatic**<br><br>If trauma please list (e.g. car crash, fall from stairs): | | Y☐ N☐ | | Y☐ N☐ | | Y☐ N☐ | | |



| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **OR** | | | | | | | | |
| 18. Was it **non-traumatic**<br><br>If non-traumatic please describe (e.g. standing up): | | Y☐ N☐ | | Y☐ N☐ | | Y☐ N☐ | | |
| 19. Current medication(s) & dose – list (if known). Other than ARV or vitamins<br><br>1._____Dose_____<br><br>2._____Dose_____<br><br>3._____Dose_____<br><br>4._____Dose_____ | | | | | | | | |
| 20. Ask if subject takes:<br><br>Calcium supplements, if Y<br><br>- List preparations_____<br><br>- Dose/number of tablets_____ | | Y☐ N☐ | | Y☐ N☐ | | Y☐ N☐ | | |
| 21. Vitamin D supplements, if Y | | Y☐ N☐ | | Y☐ N☐ | | Y☐ N☐ | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| - List preparations_____<br>- Dose/number of tablets_____ | | | | | | |
| 22. Vitamin/mineral supplements (e.g. multivitamins, BCom), if Y<br>- List preparations_____<br>- Dose/number of tablets_____ | | Y☐ N☐ | | Y☐ N☐ | | Y☐ N☐ |
| 23. History of steroid use since last visit. If Y:<br>- List preparations_____<br>- Dose/number of tablets_____ Number of months taken_____ | | Y☐ N☐ | | Y☐ N☐ | | Y☐ N☐ |
| 24. Liopodystrophy (clinician assessed) (If unsure discuss with Dr Hamill) | | Y☐ N☐ | | Y☐ N☐ | | Y☐ N☐ |
| 25. Current diarrhoea per day (if any), in past 7 days | | | | | | |
| 25. Current (most recent) CD4 count | | x | | | | |
| 26. Lowest CD4 count (if different) | | x | | | | |



| | | | | | | |
|---|---|---|---|---|---|---|
| 27. Bactrim (PCP) prophylaxis | | x | | Y☐ N☐ | | Y☐ N☐ |
| 28. Current (most recent) HIV viral load (if available) | | x | | | | |
| 29. Peak viral load, if different from previously recorded (if available) | | x | | | | |
| 30. **Current** ARV – list ARVs and start date:<br>1._____<br>Start date:<br>2._____<br>Start date:<br>3._____<br>Start date: | | | | | | |
| 31. **Previous** ARV regimes – list ARVs and start and stop dates: | | | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1._____<br>Start date: Stop date:<br><br>2._____<br>Start date: Stop date:<br><br>3._____<br>Start date: Stop date: | | | | | | | |
| 32. Cumulative duration of ALL ARV (in months) | | x | | | | | |
| 33. Cumulative duration of NRTI e.g. d4T, 3TC, AZT (in months) | | x | | | | | |
| 34. Cumulative duration of Tenofovir (TDF) (in months) | | x | | | | | |
| 35. Cumulative duration of NNRTI e.g. Efavirenz/Nevirapine (in months) | | x | | | | | |
| 36. Cumulative duration of PI e.g. Kaletra/Alluvia (in months) | | x | | | | | |
| 37. Major illness since last visit | | Y☐ N☐ | | Y☐ N☐ | | Y☐ N☐ | |



| | | | | | | | |
|---|---|---|---|---|---|---|---|
| If major illness please list what & dates<br><br>1._____<br>Start date: Stop date:<br><br>2._____<br>Start date: Stop date:<br><br>3._____<br>Start date: Stop date: | | | | | | | |
| 38. CDC stage of disease e.g. A1 | | x | | | | | |

Other comments:

Information collected by: Date:

Data captured by: Date:



## Appendix 3 Dietary assessment

Dietary intake can be assessed by a variety of methods such as weighed or estimated food records, 24-hour recalls, food frequency questionnaires (FFQ) or diet histories (Bingham *et al*, 1994, Prentice *et al*, 2009b). The method chosen depends on the characteristics of study subjects, respondent burden, study objectives and available resources. The decision for this study was to use a locally available dietary tool, a semi-quantitative FFQ, which measured habitual food intake and allowed for inclusion of traditional, locally prepared food and recently available convenience items such as fast food.

The aim was to investigate the potential role of diet on bone outcomes, in particular the role of dietary calcium intake.

### Food Frequency Questionnaire (FFQ)

Dietary intake was assessed using a FFQ based on the South African Food Composition Tables (SAFCT) developed by the Nutritional Intervention Unit of the South African Medical Research Council (MRC) (Wolmarans *et al*, 2010). Although this FFQ has not been validated against other techniques for estimating dietary intake, an early version was used to assess eating patterns and the past 6 months' food intake in children aged 1 to 9 years in the National Food Consumption Survey (Labadarios *et al*, 2005), and a modified version was then piloted in a sample of 92 adolescents from the University of Witwatersrand's Bt20 cohort and found to produce feasible estimates of energy intake (Zingoni *et al*, 2009).

There were staff trained in deploying the South African MRC FFQ and software devised to handle the data generated (MRC (SA), 2002, MacKeown *et al*, 2007, Pedro *et al*, 2008).

The food list on the questionnaire was based on an analysis of dietary surveys conducted in South African adults from 1983-2000. The list is comprehensive and includes all foods eaten by at least 3% of the population (Steyn *et al*, 2003) however it is recognized that

certain foods, such as Asian foods, are underrepresented. However is unlikely that underrepresentation of Asian dishes biased the results in the black population.

South African Food Composition Database (SAFOODS) contains nutrient information from South African origin as well as from other international databases and scientific publications. The latest 1991 edition contained 18% South African values and therefore the South African Food Composition Data (SAFCoD) steering committee was formed in 1995 to increase the South African values. This resulted in the 1998 vegetables and fruit and the 1999 milk and milk products, eggs and meat and meat products supplements. The Foodfinder 3 software was used in this study as it uses a South African food composition data and is used by nutrition researchers in South Africa (Zingoni, 2007, Wrottesley, 2011). The software in Foodfinder3 was used to calculate mean daily energy, macro- and micronutrient intakes for each individual.

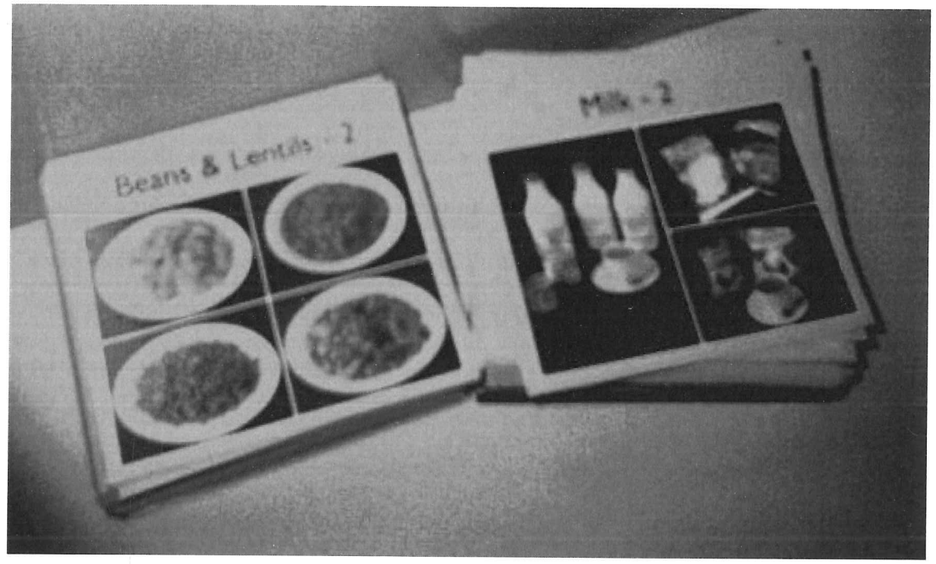
Due to the anticipated high level of illiteracy, in parts of the South African population, the FFQ had been specifically developed to make use of food flash cards; a set of over 120 flashcards representing all the foods on the questionnaire. The FFQ was administered in paper and pen format by interview with trained, multilingual members of research staff to aid communication with study participants and memory recall.

Participants were asked to separate the food flash cards into piles which differentiated between items that were eaten/drank nearly every day, less frequently, and hardly ever/never. Data were collected retrospectively about the previous seven days in order to estimate habitual intake for each participant. The FFQ captures information regarding the amount of food eaten, number of times eaten per day and portion size. Each food has an assigned code which is a unique four digit number obtained from food tables. Some additional questions on factors affecting food choice, salt use, and fast food consumption were added to the questionnaires for DPHRU studies (see questions 1-10 on the modified FFQ in Appendix 4). Figure 0-1 shows an example of the food flash cards



which are colour coded to enable quick identification of the foods in the food photo manual (FPM) e.g. dairy products are blue.

**Figure 0-1 Food flashcards used as visual aid**




The food codes were identified in the FPM and entered on the FFQ by the research scientist (AF) (see section 4.2).

The daily and frequently consumed items were then looked at individually and the participant was asked how many times per day or week the item had been consumed during the previous seven days.

### Portion size

A major challenge in with the use of FFQ is the estimation of portion size (Nelson *et al*, 1998b, 1998a, Souverein *et al*, 2011). For this study portion size was estimated using household measures, two-dimensional life-size drawings of foods and utensils, and three-dimensional food models as described below (Wrottesley, 2011).

The FPM has generic life-size sketches of food portions which show cups, mugs, glasses, bowls and spoons filled to different levels. An example is shown for a mug filled to ¼, ½, ¾ and full in (Figure 0-2). The FPM also contains 'life-size' pictures of some foods on the

reverse side of the photos in the FPM to assist in portion size determination (Zingoni, 2007).

Food models were also used for certain foods such as pap (stiff maize porridge) (Figure 0-3). Different sizes representing different size cups were used by the trained Research Assistant to assist recall and estimation of portion size.

The food pictures and food models have been shown to assess portion size with reasonable accuracy in adolescents. A study was conducted in adolescents aged 12-13 years living in Johannesburg (Steyn *et al*, 2006). The sample consisted of 50 black and 42 white children recruited from relatively low and medium income schools respectively. Each participant was presented with a plate of food of a known weight and was required to select a 2-dimensional drawing and the 3-dimensional food model which closely resembled the portion size on the plate. There were significant ($p<0.0001$) positive linear associations between the calculated nutrients using the actual and estimated portion sizes using both the pictures and the food models (Steyn *et al*, 2006, Zingoni, 2007). This provides some assurance that portion sizes would be estimated with reasonable accuracy using this tool.

### Processing of dietary intake data

The weight of the food consumed daily was calculated from the paper FFQ as follows:

**(Weight of food consumed x frequency of consumption)/time period**

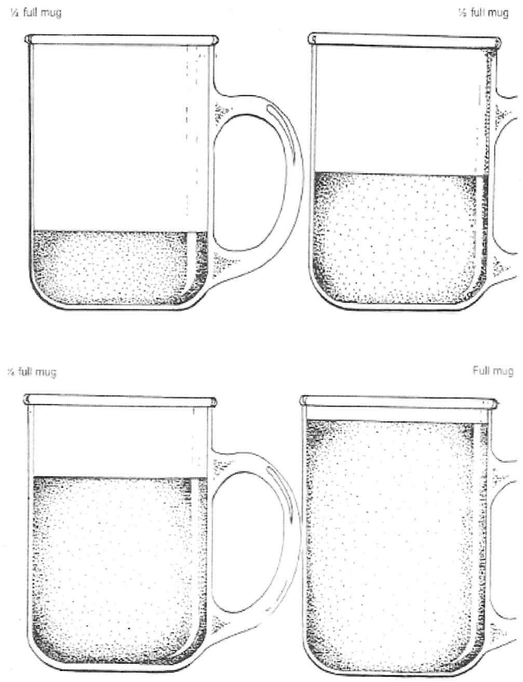
**Where time period was per day = 1, per week = 7**

After the manual calculation of daily intake of individual foods per day by a postgraduate student (SW) and a research scientist (AF) the food code was entered on the questionnaire if it had not been done during the interview. The dietary intake data were then entered into the Foodfinder 3 dietary analysis programme by SW. This is a computer software application developed by the Nutritional Intervention Research Unit and Biomedical Informatics Research Division of the South Africa MRC. This tool enables



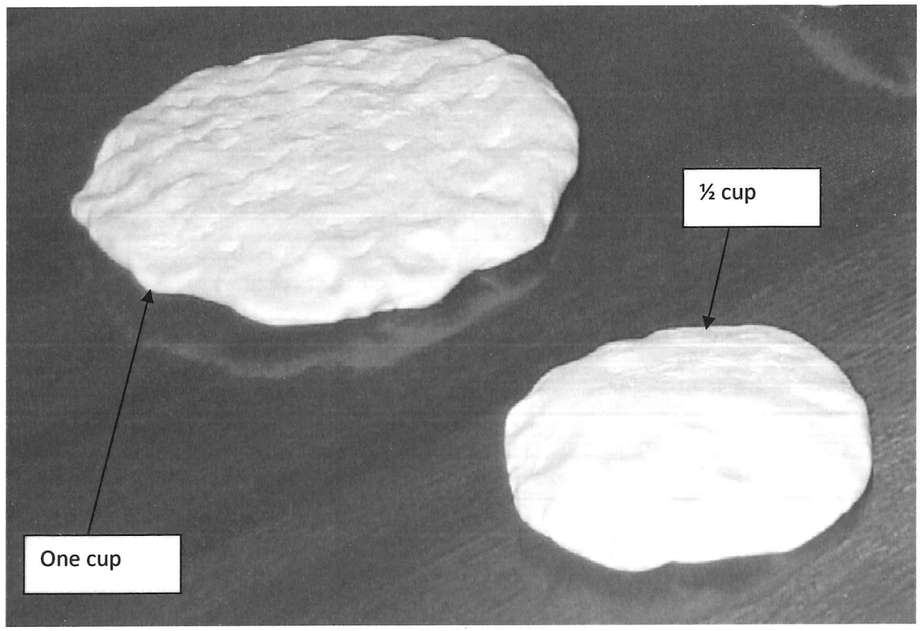
calculation of group and individual nutrient intakes. Foodfinder 3 includes the latest version of the SAFOODS with updates on vegetables and fruit and milk and milk products, eggs, meat and meat products.

**Figure 0-2 The FPM generic life-size sketches of food portions**




Not to scale

**Figure 0-3 Food flour models**



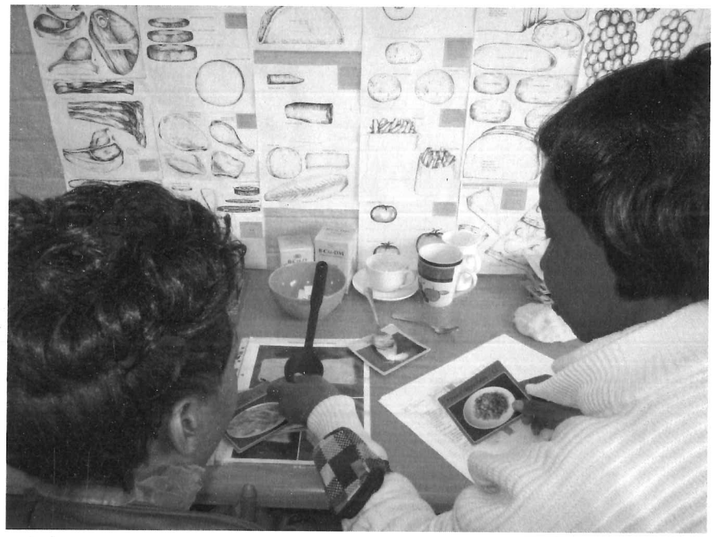

(Zingoni, 2007).

Logistic and budget constraints as well as anticipated poor literacy levels in English meant dietary assessment was best carried out when participants came to the study site for their assessments. These took approximately 3.5 hours overall therefore a dietary intake tool which did not have too great a respondent burden whilst assessing usual intake was required. It was crucial to maintain their motivation and not deter them from future visits. However, after the baseline visit, a decision was made to not repeat the FFQ at 6 and 12 month follow up visits because the FFQ took approximately 60 minutes to administer and many participants reported it to be time consuming and burdensome. As a result only baseline FFQ data were collected. At the six and 12 month study visits an amended and truncated dietary questionnaire was administered (see Appendix 5).

The set-up for the dietary intake interview is as shown in Figure 0-4.



**Figure 0-4 FFQ interview station**



The dietary data from the baseline visit can be found in, "Dietary intake and body composition in HIV-positive and -negative South African women" Submitted to *Public Health Nutrition* March 2013. Stephanie V Wrottesley, Lisa K Micklesfield, Matthew M Hamill, Gail R Goldberg, Ann Prentice, John M Pettifor, Shane A Norris and Alison B Feeley (Appendix 6).

**FOOD FREQUENCY QUESTIONNAIRE (FFQ)**

**Bone Health ID** | | | |

**Birth To Twenty ID** | | | | | | | | |

| F | M |
|---|---|

**Gender**

**Date**.....

**Interviewer's name**.....

**Food habits**

1. Are you on a special diet that has been prescribed for you e.g. by a doctor or one that you have adopted from someone e.g. a TV star/magazine?

| | |
|---|---|
| YES | 1 |
| NO | 0 |

2. If NO, go to question 4.
   If YES, describe what kind of diet you are on and where you got the diet from?

_____

3. How long have you been on that diet?_____months/years.

4. Do you currently take any vitamin and mineral supplements?

| | |
|---|---|
| YES | 1 |
| NO | 0 |

**IF YES**, what do you take?

| | Name of product | Amount/day |
|---|---|---|
| Vitamins/vitamins and minerals | | |
| Tonics | | |
| Health foods | | |
| Body building preparations | | |
| Dietary fibre supplement | | |
| Other: specify | | |

5. Which meals do you skip almost on a daily basis?

| | |
|---|---|
| Breakfast | 1 |
| Lunch | 2 |
| Evening meal | 3 |
| None | 4 |

6. Is salt added to your food while it is being cooked?

| | |
|---|---|
| Always | 1 |
| Sometimes | 2 |
| Never | 3 |
| Don't know | 4 |

7. Do you add salt to your food before you eat it?

| | |
|---|---|
| YES | 1 |
| NO | 0 |

8. If yes, how much salt do you add to your food each day?

| | |
|---|---|
| ¼ teaspoon | 1 |
| ½ teaspoon | 2 |
| ¾ teaspoon | 3 |
| 1 teaspoon | 4 |
| Other specify: | 5 |

9. Do you add Aromat to your food before you eat it?

| | |
|---|---|
| YES | 1 |
| NO | 0 |

10. If yes, how much Aromat do you add to your food each day?

| | |
|---|---|
| ¼ teaspoon | 1 |
| ½ teaspoon | 2 |
| ¾ teaspoon | 3 |
| 1 teaspoon | 4 |
| Other specify: | 5 |

11. There are some factors which influence the choice of foods we eat. Which of the following statements are true for you?

| | Strongly agree | Agree | Disagree | Strongly Disagree |
|---|---|---|---|---|
| I choose to eat certain foods because they taste good | 1 | 2 | 3 | 4 |
| The food I eat depends on whether it is expensive | 1 | 2 | 3 | 4 |
| I choose to eat certain foods because it looks good | 1 | 2 | 3 | 4 |
| The food I choose to eat differs according to my mood (i.e. happy/sad) | 1 | 2 | 3 | 4 |
| My hunger level determines what type of food I eat. | 1 | 2 | 3 | 4 |
| I choose foods which are not time consuming to prepare | 1 | 2 | 3 | 4 |
| I consider whether a food is good for my health before eating the food. | 1 | 2 | 3 | 4 |

12. Do you ever eat outside the home e.g. at fast food shops such as Nandos, KFC and Steers?

| | |
|---|---|
| YES | 1 |
| NO | 0 |

13. If YES, in an average month how often do you eat at the following places?

| | Frequency of visits | | |
|---|---|---|---|
| | Times/week | Times/month | Rarely/never |
| Nandos | | | |
| Spur | | | |
| Macdonalds | | | |
| Steers | | | |
| KFC | | | |
| Chicken Licken | | | |
| Debonaire's Pizza | | | |
| Romans | | | |
| Anat | | | |
| Wimpy | | | |
| Something fishy | | | |
| Fontana | | | |
| Chinese takeaway | | | |
| Other restaurants/takeaways | | | |
| (Quarters from tuck shop) | | | |
| | | | |



| Generic Sketch (look up) | A. Food items (with FPM numbers) | B. Description of food item | Tick for yes | C. Item code | D. Amount usually eaten(g) Generic/amount = g | E. Eaten every day Times/day | F. Eaten every week Times/week | G. Eaten Occasionally | H. Never eaten |
|---|---|---|---|---|---|---|---|---|---|
| | DAIRY-BLUE | | | | | | | | |
| | **1.Tea** | Ordinary | | 4038 | | | | | |
| | | Herbal | | 4053 | | | | | |
| | | Rooibos | | 4054 | | | | | |
| | **1. Sugar in tea** | | | 3989 | | | | | |
| | **2. Milk in tea** | Full cream | | | | | | | |
| | | Low fat 2% | | | | | | | |
| | | Skim fat free | | | | | | | |
| | | Other | | | | | | | |
| | **1. Coffee** | | | 4037 | | | | | |
| | **2. Milk in coffee** | Full cream | | | | | | | |
| | | Low fat 2% | | | | | | | |
| | | Skim fat free | | | | | | | |
| | | Other | | | | | | | |
| | **2. Sugar in coffee** | | | 3989 | | | | | |
| | **2. Milk as a drink** | Full cream | | | | | | | |
| | | Low fat 2% | | | | | | | |
| | | Skim fat free | | | | | | | |
| | **3. Buttermilk/maas** | Buttermilk | | 2713 | | | | | |
| | | Maas | | 2787 | | | | | |
| | **4. Milk drinks, flavoured** | | | | | | | | |
| X | **5. Yoghurt** | Flavoured | | | | | | | |
| | | Plain | | | | | | | |
| | **1. Sugar (extra)** | | | | | | | | |

| **Generic Sketch** (look up) | **A.** Food items (with FPM numbers) | **B.** Description of food item | **Tick for yes** | **C.** Item code | **D.** Amount usually eaten(g) Generic/amount = g | **E.** Eaten every day | **F.** Eaten every week | **G.** Eaten Occasionally | **H.** Never eaten |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Times/ day | Times/ week | | |
| | **3. Breakfast cereals** | | | | | | | | |
| | **2. Milk added to cereal** | | | | | | | | |
| | **1. Sugar added to cereal** | | | | | | | | |
| | **9. Ice cream** | Full cream | | | | | | | |
| | | Low sugar | | | | | | | |
| | **9. Ice lollies** | | | | | | | | |
| | **ASK ABOUT TYPE OF BREAD,THICKNESS OF SLICES AND THE SPREAD** | | | | | | | | |
| | **1. Bread/rolls** | White | | | | | | | |
| | | Brown | | | | | | | |
| | | Whole wheat | | | | | | | |
| | | Traditional | | | | | | | |
| | | Roti | | | | | | | |
| | **Spread? Y / N** | **Brand name** | | | | | | | |
| | **1. Brick margarine** | | | | | | | | |
| | **1. Tub margarine** | | | | | | | | |
| | **1. Butter** | | | | | | | | |
| | **Cheese spread** | | | | | | | | |
| | **Fish paste** | | | | | | | | |
| | **Honey/syrup** | | | | | | | | |
| | **Jam** | | | | | | | | |
| | **Marmite/Bovril** | | | | | | | | |
| | **Sandwich spread** | | | | | | | | |
| | **Peanut butter** | | | | | | | | |
| | **Chocolate spread** | | | | | | | | |



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|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Times/ day | Times/ week | | |
| | | | | | | | | | |
| | **Cold meats** | Ham | | | | | | | |
| | | Polony | | | | | | | |
| | | Salami | | | | | | | |
| | **6. Cottage cheese** | Low fat | | | | | | | |
| | | Full fat | | | | | | | |
| | **7. Cheddar** | | | | | | | | |
| | **7. Gouda** | | | | | | | | |
| | **7. Other cheese** | | | | | | | | |
| | **8. Cheese wedges** | | | | | | | | |
| | **2. Fat cakes** | | | | | | | | |
| (Red) | **QUARTER** | | | | | | | | |
| | | | | | | | | | |
| | **4. Maize porridge stiff** | | | | | | | | |
| | **4. Maize porridge soft** | | | | | | | | |
| | 2. Milk on soft porridge | | | | | | | | |
| | 1. Sugar on soft porridge | | | | | | | | |
| | 1. Fat on soft porridge | | | | | | | | |
| | **4. Mabele/maltabella stiff** | | | | | | | | |
| | **4. Mabele soft** | | | | | | | | |
| | 2. Milk on Mabele | | | | | | | | |
| | 1. Sugar on Mabele | | | | | | | | |
| | 1. Fat on Mabele | | | | | | | | |
| | **4. Oats** | | | | | | | | |
| | 2. Milk on Oats | | | | | | | | |
| | 1. Sugar on Oats | | | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Times/ day | Times/ week | | |
| | **4. Maize & pumpkin porridge** | | | | | | | | |
| | **5. Pasta/Pasta dishes** | | | | | | | | |
| | | | | | | | | | |
| | **6. Spaghetti Bolognaise** | | | | | | | | |
| | **6. Macaroni and cheese** | | | | | | | | |
| | **6. Lasagne** | | | | | | | | |
| | **7. Rice** | White | | | | | | | |
| | | Brown | | | | | | | |
| | | With fat? | | | | | | | |
| | **7. Samp/mealie rice** | With fat? | | | | | | | |
| | **7. Samp and beans** | With fat? | | | | | | | |
| X | **7. Wheat rice** | With fat? | | | | | | | |
| | **8. Pizza** | Vegetable topping | | | | | | | |
| | | Meat topping | | | | | | | |
| | | Meat & vegetables | | | | | | | |
| | **8. Savoury tart** | | | | | | | | |
| X | **EGGS-YELLOW** | | | | | | | | |
| | **Boiled** | | | | | | | | |
| | **Fried** | | | | | | | | |
| | **Omelette** (filling and quantity) | | | | | | | | |
| | **Scrambled** | | | | | | | | |
| **Generic Sketch** | **A.** Food items (with FPM | **B.** Description of | **Tick for** | **C.** Item | **D.** Amount usually | **E.** Eaten | **F.** Eaten | **G.** Eaten | **H.** Never |



| (look up) | numbers) | food item | yes | code | eaten(g) Generic/amount = g | every day | every week | Occasionally | eaten |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Times/ day | Times/ week | | |
| | **FRUITS-ORANGE** | | | | | | | | |
| X | **1. Apples** | | | | | | | | |
| X | **2. Bananas** | Fresh | | | | | | | |
| | | Fried | | | | | | | |
| | **3. Berries** | Fresh | | | | | | | |
| | | Canned | | | | | | | |
| | | Candied | | | | | | | |
| X | **4. Figs/prickly pears &** | Fresh | | | | | | | |
| | **Coconuts, dates** | Candied | | | | | | | |
| | **5. Fruit salad** | Fresh | | | | | | | |
| | | Canned juice | | | | | | | |
| | | Canned syrup | | | | | | | |
| X | **6. Grapes** | | | | | | | | |
| X | **7. Guavas** | Fresh | | | | | | | |
| | | Canned juice | | | | | | | |
| | | Canned syrup | | | | | | | |
| X | **8. Mango** | Fresh | | | | | | | |
| | | Canned juice | | | | | | | |
| | | Canned syrup | | | | | | | |
| | **8. Pawpaw** | Fresh | | | | | | | |
| | | Canned juice | | | | | | | |
| | | Canned syrup | | | | | | | |
| | **8. Kiwi fruit** | | | | | | | | |
| | **8. Leechies** | | | | | | | | |
| X | **9. Watermelons** | | | | | | | | |
| | **9. Sweet melon** | | | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Times/ day | Times/ week | | |
| X | **10. Naartjies** | Mineola fresh | | 4227 | | | | | |
| | | Naartjie fresh | | 3558 | | | | | |
| | | Naartjie canned in syrup | | 3635 | | | | | |
| X | **11. Oranges** | Fresh | | 3560 | | | | | |
| | **11. Grapefruit** | Fresh | | 3546 | | | | | |
| X | **12. Peaches** | Fresh | | | | | | | |
| | | Canned juice | | | | | | | |
| | | Canned syrup | | | | | | | |
| X | **12. Nectarines** | Fresh | | | | | | | |
| X | **13. Pears** | Fresh | | | | | | | |
| | | Canned juice | | | | | | | |
| | | Canned syrup | | | | | | | |
| | **14. Pineapple** | Fresh | | | | | | | |
| | | Canned juice | | | | | | | |
| | | Canned syrup | | | | | | | |
| X | **15. Plums** | Fresh | | | | | | | |
| | **15. Apricots** | | | | | | | | |
| | **16. Dried fruit** | | | | | | | | |
| | **16. Dry stewed fruit** | | | | | | | | |
| | **16. Raisins** | | | | | | | | |
| | **17. Fruit juice** | Fresh | | | | | | | |
| | | Sweetened | | | | | | | |
| | | Unsweetened | | | | | | | |


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|---|---|---|---|---|---|---|---|---|---|




| | | | | | Generic/amount = g | day | week | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Times/ day | Times/ week | | |
| | **SOUPS, LEGUMES, NUTS** | | | | | | | | |
| | **1. Soups** | Homemade | | | | | | | |
| | | Commercial | | | | | | | |
| | | Vegetable | | | | | | | |
| | | Meat | | | | | | | |
| | | Meat & vegetable | | | | | | | |
| | | With bones | | | | | | | |
| | **2. Beans and lentils** | Anything added | | | | | | | |
| | **3. Nuts** | | | | | | | | |
| | **3. Peanuts** | | | | | | | | |
| | **FISH & SEAFOOD- BEIGE** | | | | | | | | |
| | **1. Fried fish** | | | | | | | | |
| | **1. Fish cakes** | | | | | | | | |
| | **1. Fish fingers** | | | | | | | | |
| | **1. Calamari** | | | | | | | | |
| | **2. Grilled/smoked/dried fish** | | | | | | | | |
| | **2. Haddock** | | | | | | | | |
| | **3. Pilchards & sardines** | Canned water | | | | | | | |
| | | Canned tomato sauce | | | | | | | |
| | | Mayonnaise | | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Times/ day | Times/ week | | |
| | **3. Tuna** | In brine water | | | | | | | |
| | | In oil | | | | | | | |
| | | Mayonnaise | | | | | | | |
| | **3. Pickled fish** | | | | | | | | |
| | **MEAT- RED** | | | | | | | | |
| | How do you eat meat 1= with fat 2= fat trimmed | | | | | | | | |
| | **1. Roast beef** | | | | | | | | |
| | **1. Beef chops** | | | | | | | | |
| | **1. Beef steak (bone)** | | | | | | | | |
| | **1. Beef steak (no bone)** | | | | | | | | |
| | **1. Beef stir-fry** | | | | | | | | |
| | **1. Beef stew / carrots** | | | | | | | | |
| | **1. Beef stew / cabbage** | | | | | | | | |
| | **2. Beef patties** | | | | | | | | |
| | **2. Mince** | Regular | | | | | | | |
| | | Lean | | | | | | | |
| | | With fat | | | | | | | |
| | | With vegetables | | | | | | | |
| X | **2. Meatballs** | | | | | | | | |
| | **2. Cottage pie** | | | | | | | | |
| | **3. Burgers** | Homemade | | | | | | | |
| | | Fried | | | | | | | |
| | | Takeaway | | | | | | | |
| | | Crumbed | | | | | | | |
| | **3. Hot dog** | | | | | | | | |
| | **3. Pita with…** | | | | | | | | |



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|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Times/ day | Times/ week | | |
| | **3. Chicken burger** | Crumbed | | | | | | | |
| | | Not crumbed | | | | | | | |
| | | Takeaway | | | | | | | |
| | | Home made | | | | | | | |
| | | Shop bought | | | | | | | |
| | **3. Chicken nuggets** | | | | | | | | |
| | **4. Chicken stew** | | | | | | | | |
| | **4. Fried chicken pieces** | With skin | | | | | | | |
| | | Without skin | | | | | | | |
| | **4. Roast chicken** | With skin | | | | | | | |
| | | Without skin | | | | | | | |
| | **4. Chicken stir-fry** | | | | | | | | |
| | **4. Chicken schnitzel** | | | | | | | | |
| [1] | | | | | | | | | |
| | **6. Fat cake & mince** | | | | | | | | |
| | **7. Meat pies** | Home made | | | | | | | |
| | **King pie / pie city** | Commercial | | | | | | | |
| | | | | | | | | | |
| | **7. Samoosas** | Meat | | | | | | | |
| | | Vegetable | | | | | | | |
| | **7. Sausage rolls** | | | | | | | | |
| | **7. Spring rolls** | | | | | | | | |

| Generic Sketch (look up) | A. Food items (with FPM numbers) | B. Description of food item | Tick for yes | C. Item code | D. Amount usually eaten(g) | E. Eaten every | F. Eaten every | G. Eaten Occasionally | H. Never eaten |
|---|---|---|---|---|---|---|---|---|---|

[1] See BLUE for 5 cold meats

| | | | | | Generic/amount = g | day | week | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Times/ day | Times/ week | | |
| | **8. Mutton stew no veg** | | | | | | | | |
| | **8. Mutton stew with veg** | | | | | | | | |
| | **8. Mutton leg chop** | | | | | | | | |
| | **8. Mutton loin chop** | | | | | | | | |
| | **8. Roast mutton** | | | | | | | | |
| | **9. Spare ribs** | | | | | | | | |
| | **9. Pork chops** | | | | | | | | |
| | **9. Bacon** | | | | | | | | |
| | **9. Roast pork** | | | | | | | | |
| | **10. Pork sausages** | | | | | | | | |
| | **10. Viennas** | | | | | | | | |
| | **10. Frankfurters** | | | | | | | | |
| | **10. Boerewors** | | | | | | | | |
| | **11. Traditional / organ meats** | | | | | | | | |
| | **Chicken livers** | | | | | | | | |
| | **Chicken organ meats** | | | | | | | | |
| | **Chicken head & feet** | | | | | | | | |
| | **Liver & fat** | | | | | | | | |
| | **Sheep intestine / lungs** | | | | | | | | |
| | **Pork shank** | | | | | | | | |
| | **Mopani worms** | | | | | | | | |
| | | | | | | | | | |



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|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Times/ day | Times/ week | | |
| | **12. Vegetarian products** | | | | | | | | |
| | **13. Dry sausages** | | | | | | | | |
| | **13. Biltong** | | | | | | | | |
| | **VEGETABLES – GREEN** | | | | | | | | |
| | **1. Asparagus** | Fresh | | | | | | | |
| | | Canned | | | | | | | |
| | | Fat added | | | | | | | |
| | | Sugar added | | | | | | | |
| | **2. Avocado** | Fresh | | | | | | | |
| | **3. Baby marrows** | Fat added | | | | | | | |
| | | Sugar added | | | | | | | |
| | | Sauce | | | | | | | |
| | **4. Beetroot** | Boiled | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | **5. Butternut/pumpkin** | Fat added | | | | | | | |
| | | Sugar added | | | | | | | |
| | **6. Broccoli** | Fat added | | | | | | | |
| | **6. Cauliflower** | Fat added | | | | | | | |
| | **7. Cabbage** | Boiled | | | | | | | |
| | | Fried | | | | | | | |
| | **8. Carrots** | Boiled | | | | | | | |
| | | Fat added | | | | | | | |
| | | Sugar added | | | | | | | |

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|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Times/ day | Times/ week | | |
| | **9. Gem squash** | Boiled | | | | | | | |
| | | Sugar added | | | | | | | |
| | | Sauce | | | | | | | |
| | **10. Green beans** | Fat added | | | | | | | |
| | | Sugar added | | | | | | | |
| | | With onion | | | | | | | |
| | | With potato | | | | | | | |
| | | Sauce | | | | | | | |
| | **11. Mealies** | Fat added | | | | | | | |
| | | Creamed | | | | | | | |
| | **12. Mixed vegetables** | Frozen | | | | | | | |
| | | Fat added | | | | | | | |
| | | Sugar added | | | | | | | |
| | | Sauce | | | | | | | |
| | **13. Mushrooms** | Fat added | | | | | | | |
| | | Sauce | | | | | | | |
| | **14. Peas** | Fat added | | | | | | | |
| | | Sauce | | | | | | | |
| | **15. Potatoes** | Boiled | | | | | | | |
| | | Roasted | | | | | | | |
| | | Baked | | | | | | | |
| | | Mash with fat | | | | | | | |



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|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Times/ day | Times/ week | | |
| | **15. Potato Salad** | | | | | | | | |
| | **16. Potato chips** | Homemade | | | | | | | |
| | | Fried oil | | | | | | | |
| | | Oven baked | | | | | | | |
| | | Takeaway | | | | | | | |
| | | Mayonnaise | | | | | | | |
| | | Tomato sauce | | | | | | | |
| | **17. Salad vegetables** | Mayonnaise | | | | | | | |
| | | Dressing | | | | | | | |
| | **17. Cucumber** | | | | | | | | |
| | **17. Peppers** | | | | | | | | |
| | **18. Spinach/morogo** | Fat added | | | | | | | |
| | | Sauce added | | | | | | | |
| | | With onions | | | | | | | |
| | | With potatoes | | | | | | | |
| | **19. Sweet potatoes** | Fat added | | | | | | | |
| | | Sugar added | | | | | | | |
| | **20. Tomatoes** | Raw | | | | | | | |
| | | Cooked | | | | | | | |
| | | With onion | | | | | | | |
| | | Fat added | | | | | | | |

| **Generic Sketch** (look up) | **A.** Food items (with FPM numbers) | **B.** Description of food item | **Tick for yes** | **C.** Item code | **D.** Amount usually eaten(g) Generic/amount = g | **E.** Eaten every day | **F.** Eaten every week | **G.** Eaten Occasionally | **H.** Never eaten |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Times/ day | Times/ week | | |
| | **FATS-TAN 'prompt'** | | | | | | | | |
| | **1. Tub margarine** | Where used | | | | | | | |
| | | Number of spoons | | | | | | | |
| | | Number in family | | | | | | | |
| | **1, Butter** | | | | | | | | |
| | **1. Brick Margarine** | | | | | | | | |
| | **2. White margarine** (type of fat) | | | | | | | | |
| | **3. Cream and substitutes** (brand, real dairy/plant fats) | | | | | | | | |
| | **4. Oils** | Where used | | | | | | | |
| | | Number of spoons | | | | | | | |
| | | Number in family | | | | | | | |
| | **5. Salad dressings** | Homemade | | | | | | | |
| | | Shop bought | | | | | | | |
| | | | | | | | | | |
| | **5. Mayonnaise** | Where used | | | | | | | |
| | | Number of spoons | | | | | | | |
| | | Number in family | | | | | | | |



| **Generic Sketch** (look up) | **A.** Food items (with FPM numbers) | **B.** Description of food item | **Tick for yes for yes** | **C.** Item code | **D.** Amount usually eaten(g) Generic/amount = g | **E.** Eaten every day | **F.** Eaten every week | **G.** Eaten Occasionally | **H.** Never eaten |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Times/ day | Times/ week | | |
| | **BISCUITS, CAKE & PUDDING - PURPLE** | | | | | | | | |
| | **1. Biscuits/cookies** | Homemade | | | | | | | |
| | | Shop bought | | | | | | | |
| | **2. Biscuits/savoury** | | | | | | | | |
| | | Spread | | | | | | | |
| | | | | | | | | | |
| | **3. Special buns** | Spread | | | | | | | |
| | **3. Muffins** | Spread | | | | | | | |
| | **3. Scones** | Spread | | | | | | | |
| | **4. Tart** | | | | | | | | |
| | **4. Cake** | Iced/Cream | | | | | | | |
| | | Plain | | | | | | | |
| | **5. Doughnuts** | Plain | | | | | | | |
| | **5. Doughnuts** | Filled | | | | | | | |
| | **5. éclairs** | | | | | | | | |
| | **5. Koeksisters** | | | | | | | | |
| | **6. Pancakes/crumpets** | Spread | | | | | | | |
| | | Syrup | | | | | | | |
| | | Ice-cream | | | | | | | |
| | **6. Waffles** | Spread | | | | | | | |
| | | Syrup | | | | | | | |
| | | Ice-cream | | | | | | | |
| | **7. Trifle** | | | | | | | | |
| | **7. Baked Pudding** | | | | | | | | |
| | **7. Instant pudding** | | | | | | | | |
| | **7. Custard** | | | | | | | | |
| | **8. Rusks** | | | | | | | | |

| Generic Sketch (look up) | A. Food items (with FPM numbers) | B. Description of food item | Tick for yes for yes | C. Item code | D. Amount usually eaten(g) Generic/amount = g | E. Eaten every day | F. Eaten every week | G. Eaten Occasionally | H. Never eaten |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Times/ day | Times/ week | | |
| | **9. Special breads** | | | | | | | | |
| | | Spread | | | | | | | |
| | **SNACKS, SWEETS & COLD DRINKS-PINK** | | | | | | | | |
| | **1. Carbonated cold drinks** e.g. coke | | | | | | | | |
| | **1. Diet cold drinks** e.g. diet coke | | | | | | | | |
| | **2. Mageu** | | | | | | | | |
| | **2. Cold drinks (powder)** | | | | | | | | |
| | **2. Energy drinks** | | | | | | | | |
| | **2. Squashes** | | | | | | | | |
| | **3. Crisps** | | | | | | | | |
| | **3. Popcorn** | | | | | | | | |
| | **4. Sweets** | | | | | | | | |
| | **Lollipops** | | | | | | | | |
| | **4. Chocolates** | | | | | | | | |
| | | | | | | | | | |
| | **SAUCES & CONDIMENTS-GREY** | | | | | | | | |
| | **1. Cheese sauce** | Full cream milk | | | | | | | |
| | | Butter | | | | | | | |
| | | Margarine | | | | | | | |
| | **1. White sauce** | Full cream milk | | | | | | | |
| | | Butter | | | | | | | |
| | | Margarine | | | | | | | |



| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|

| Generic Sketch (look up) | A. Food items (with FPM numbers) | B. Description of food item | Tick for yes for yes | C. Item code | D. Amount usually eaten(g) Generic/amount = g | E. Eaten every day | F. Eaten every week | G. Eaten Occasionally | H. Never eaten |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Times/ day | Times/ week | | |
| | **2. Chakalaka** | | | | | | | | |
| | **2. Atjar** | | | | | | | | |
| | **2. Tomato sauce & other** | | | | | | | | |
| | **2. Chutney** | | | | | | | | |
| | **ALCOHOLIC DRINKS-GREY** | | | | | | | | |
| | **1. Beer** (regular/low alcohol) | | | | | | | | |
| | **1. Cider** | | | | | | | | |
| | **Coolers** | | | | | | | | |
| | **2. Wine** | | | | | | | | |
| | **2. Champagne** | | | | | | | | |
| | **3. Spirits** (any carbonated drink e.g. coke added) | | | | | | | | |
| | | | | | | | | | |
| | **4. Liqueurs & Fortified wine** | | | | | | | | |
| | **OTHER** | | | | | | | | |

**Women's Bone Study (WBS) dietary assessment**

**Study ID** | | | | | | |

**Visit (circle)**

| 6 month | 12 month | 24 month |
|---|---|---|

**Date**……..

**Interviewer's name**……..

***THESE QUESTIONS RELATE TO YOUR FOOD HABITS SINCE WE LAST SAW YOU***
**Food habits**

1. Are you on a special diet that has been prescribed for you e.g. by a doctor or one that you have adopted from someone e.g. a TV star/magazine?

| | |
|---|---|
| YES | 1 |
| NO | 0 |

If **YES** Where did you get this advice to change your diet (tick all that apply)?

| | Tick |
|---|---|
| Magazine/TV/radio/internet | |
| Friend/Family | |
| Clinic/Hospital: | |
| 1. Doctor | |
| 2. Nurse | |
| 3. Dietician | |
| My own decision | |
| Other – please write in: | |

Describe what kind of diet you are on:

_____

Does it involve excluding certain foods, if so what?_____
Does it involve including certain foods, if so what? _____

2. If **NO**, go to question 4.

3. How long have you been on that diet?_____weeks/months/years.

4. Do you currently take any vitamin and mineral or other dietary supplements?

| | |
|---|---|
| YES | 1 |
| NO | 0 |

**IF YES**, what do you take?

| | Name of product | Amount/day (or how many tablets) |
|---|---|---|
| Vitamins/vitamins and minerals | | |
| Tonics | | |
| Vitamin D only | | |
| Calcium only | | |
| Body building preparations | | |
| Dietary fibre supplement | | |
| Other: specify | | |



5. Which meals do you skip almost on a daily basis?

| | |
|---|---|
| Breakfast | 1 |
| Lunch | 2 |
| Evening meal | 3 |
| None | 4 |

6. Is salt added to your food while it is being cooked?

| | |
|---|---|
| Always | 1 |
| Sometimes | 2 |
| Never | 3 |
| Don't know | 4 |

7. Do you add salt to your food before you eat it?

| | |
|---|---|
| YES | 1 |
| NO | 0 |

8. If yes, how much salt do you add to your food each day?

| | |
|---|---|
| ¼ teaspoon | 1 |
| ½ teaspoon | 2 |
| ¾ teaspoon | 3 |
| 1 teaspoon | 4 |
| Other specify: | 5 |

9. Do you add Aromat to your food before you eat it?

| | |
|---|---|
| YES | 1 |
| NO | 0 |

10. If yes, how much Aromat do you add to your food each day?

| | |
|---|---|
| ¼ teaspoon | 1 |
| ½ teaspoon | 2 |
| ¾ teaspoon | 3 |
| 1 teaspoon | 4 |
| Other specify: | 5 |

11. There are some factors which influence the choice of foods we eat. Which of the following statements are true for you?

| | Strongly agree | Agree | Disagree | Strongly Disagree |
|---|---|---|---|---|
| I choose to eat certain foods because they taste good | 1 | 2 | 3 | 4 |
| The food I eat depends on whether it is expensive | 1 | 2 | 3 | 4 |
| I choose to eat certain foods because it looks good | 1 | 2 | 3 | 4 |
| The food I choose to eat differs according to my mood (i.e. happy/sad) | 1 | 2 | 3 | 4 |
| My hunger level determines what type of food I eat. | 1 | 2 | 3 | 4 |
| I choose foods which are not time consuming to prepare | 1 | 2 | 3 | 4 |
| I consider whether a food is good for my health before eating the food. | 1 | 2 | 3 | 4 |

12. Do you ever eat outside the home e.g. at fast food shops such as Nandos, KFC and Steers?

| | |
|---|---|
| YES | 1 |
| NO | 0 |

13. If YES, in an average month how often do you eat at the following places?

| | Frequency of visits | | |
|---|---|---|---|
| | Times/week | Times/month | Rarely/never |
| Nandos | | | |
| Spur | | | |
| Macdonalds | | | |
| Steers | | | |
| KFC | | | |
| Chicken Licken | | | |
| Debonaire's Pizza | | | |
| Romans | | | |
| Anat | | | |
| Wimpy | | | |
| Something fishy | | | |
| Fontana | | | |
| Chinese takeaway | | | |
| Other restaurants/takeaways | | | |
| (Quarters from tuck shop) | | | |

| | |
|---|---|
| YES | 1 |
| NO | 0 |



14. Have you made any changes to your diet since your last visit?
(***Double check*** *that there are no changes in appetite, seasonal foods etc.*)

If **No** then go to question 15. (***Double check*** *that there are no changes in appetite, seasonal foods etc.*)

If **Yes**, are you eating:

| | | | | | |
|---|---|---|---|---|---|
| **More** than before? *or* | Y 1 / N 0 | If Y, is it more often? | Y 1 / N 0 | Is it bigger portions? | Y 1 / N 0 |
| **Less** than before? | Y 1 / N 0 | If Y, is it less often? | Y 1 / N 0 | Is it smaller portions? | Y 1 / N 0 |
| Are you eating foods you have never eaten before? | Y 1 / N 0 | If Y, which foods: 1.____ 2.____ 3.____ | Notes (why): | | |
| Have you stopped eating foods you used to eat? | Y 1 / N 0 | If Y, which foods: 1.____ 2.____ 3.____ | Notes (why): | | |
| Food that is more available in certain seasons? (e.g mango) | Y 1 / N 0 | If Y, which foods: 1.____ 2.____ 3.____ | Notes: | | |
| Have you changed the amount that you snack? | Y 1 / N 0; If Y, is it: More 1 / Less 0 | If Y, which foods: 1.____ 2.____ 3.____ | Notes (why): | | |

| | | | | | |
|---|---|---|---|---|---|
| **For subjects taking ARTs:** If you have started ART are you getting nausea or sickness? | Y 1 / N 0; Y 1 / N 0 | If Y, has this changed the way you eat? | Y 1 / N 0 | If Y, do you eat: More Y 1 / N 0 **or** Less Y 1 / N 0 | Which foods, if any, do you avoid? 1.____ 2.____ 3.____ Which foods, if any, do you eat more of? 1.____ 2.____ 3.____ |

15. Has your appetite changed since your last visit?

| | |
|---|---|
| YES | 1 |
| NO | 0 |

If **Yes** has it increased or decreased?

| | |
|---|---|
| Increased | 1 |
| Decreased | 0 |

Any other information about changes in diet since last visit:_____

_____

16. Alcohol

| | |
|---|---|
| Y | 1 |
| N | 0 |

Do you drink alcohol?

If **Yes**, how much in an average week

| Type of alcohol | Brand name | Amount e.g. 1 small glass or bottle | Number of times per week e.g. 3 days |
|---|---|---|---|
| Beer | | | |
| Cider | | | |
| Coolers (e.g. Storm) | | | |
| Wine | | | |
| Champagne | | | |
| Spirits | | | |
| Liqueurs & fortified wine | | | |
| Other | | | |

Has the amount you drink changed since your last visit?

| | |
|---|---|
| Y | 1 |
| N | 0 |

If **Yes**, has it increased or decreased?

| | |
|---|---|
| Increased | 1 |
| Decreased | 0 |

Any other comments_____

_____

Checked by: ..... Date.....

Data entry by: .....Date.....





**Title: Dietary intake and body composition in HIV-positive and -negative South African women**

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Running title: HIV, diet and body composition in SA women

Keywords: HIV, diet, body composition, obesity

**Acknowledgements:**

Study was designed by MMH and AP and study design reviewed by MMH, JMP, SAN and AP.

SVW and ABF analysed data. The initial phase of data analysis was conducted as part of SW's MSc research project at the London School of Hygiene and Tropical Medicine.

All authors contributed to interpretation and the writing of the manuscript.

All authors had full access to the data. A P, JMP and SAN had responsibility for the final decision to submit the manuscript for publication.

All authors report no conflicts of interest

16. Alcohol

| | |
|---|---|
| Y | 1 |
| N | 0 |

Do you drink alcohol?

If **Yes**, how much in an average week

| Type of alcohol | Brand name | Amount e.g. 1 small glass or bottle | Number of times per week e.g. 3 days |
|---|---|---|---|
| Beer | | | |
| Cider | | | |
| Coolers (e.g. Storm) | | | |
| Wine | | | |
| Champagne | | | |
| Spirits | | | |
| Liqueurs & fortified wine | | | |
| Other | | | |

Has the amount you drink changed since your last visit?

| | |
|---|---|
| Y | 1 |
| N | 0 |

If **Yes**, has it increased or decreased?

| | |
|---|---|
| Increased | 1 |
| Decreased | 0 |

Any other comments_____

_____

Checked by: …….. Date……..

Data entry by: ……Date……..





**Title: Dietary intake and body composition in HIV-positive and -negative South African women**

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All authors contributed to interpretation and the writing of the manuscript.

All authors had full access to the data. A P, JMP and SAN had responsibility for the final decision to submit the manuscript for publication.

All authors report no conflicts of interest

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**Abstract**

*Objective:* This paper examines dietary intake and body composition in ARV-naïve HIV-positive compared to HIV-negative SA women, as well as the impact of disease severity on these variables.

*Design:* Baseline data from a longitudinal study assessing bone health in HIV-negative and HIV-positive pre-menopausal SA women over 18 years of age were used. Anthropometry and body composition, measured by dual energy X-ray absorptiometry (DXA), were analysed together with dietary intake data assessed using an interviewer based qualitative food frequency questionnaire (FFQ).

*Setting:* Soweto, Johannesburg, South Africa

*Subjects:* Black, urban SA women were divided into three groups: HIV-negative (n=98), HIV-positive with preserved $CD_4$ counts (HIV+ non-ARV; n=74) and HIV-positive with low $CD_4$ counts due to start ARV treatment (HIV+ pre-ARV; n=75).

*Results:* The prevalence of overweight and obesity was high in this population (59%). The HIV+ pre-ARV group was lighter and had a lower BMI than the other two groups (all $p<0.001$). Pre-ARV subjects also had lower fat and lean masses and % body fat than their HIV-negative and HIV+ non-ARV counterparts. After adjustment, there were no differences in macronutrient intakes across study groups; however, fat and sugar intakes were high and consumption of predominantly refined food items was common overall.

*Conclusion:* HIV-associated immunosuppression may be a key determinant of body composition in HIV-positive women. However, in populations with high obesity prevalence, these differences become evident only at advanced stages of infection.



## Introduction

The introduction of antiretroviral (ARV) therapy has dramatically altered the morbidity profile of HIV-positive populations, with an increase in prevalence of non-communicable disease (NCD) risk factors such as overweight and obesity being observed[1;2]. This is of particular concern in South Africa where, amongst adults over 15 years of age, 17.8% were estimated to be living with HIV in 2008[3] and approximately 45% were found to be overweight or obese in the 2003 Demographic and Health Survey (SADHS)[4].

Although the public health impact of HIV and associated NCD risk is greatest in low-middle-income countries (LMICs), to date most studies have been conducted in high-income countries, on predominantly ARV-treated male subjects with low BMIs[5-8]. Lipodystrophy, a commonly recognized side-effect of certain ARV drugs, has been linked to visceral fat accumulation and metabolic disorders such as dyslipidaemia and glucose intolerance[9]. In black SA women ARV-associated increases in body mass index (BMI), fat mass, body fat percentage and waist circumference, but not in lean body mass or waist: hip ratio, have been shown[10]. This increase in fat, as well as, the proposed tendency towards visceral rather than subcutaneous fat accumulation[11], must therefore be further explored in this population due to the potential long-term negative-effects on disease risk and health[12].

While the evidence for body composition changes associated with ARV drugs continues to grow, there is less research focusing on the effect of the HIV infection itself. Hadigan et al[13] found that, independent of ARV, HIV-positive women in the USA demonstrated a higher percentage body fat and truncal fat and a lower percentage lean body mass than HIV-negative controls. In addition, other data suggest that HIV-positive patients with higher BMIs, skinfold thicknesses and insulin concentrations prior to ARV initiation may be more likely to develop lipodystrophy over 2 years of ARV drug use[14].

Although HIV-positive status has traditionally been associated with low energy intake, most likely as a result of reduced appetite[15], SA data show energy intake in HIV-positive subjects to be equivalent to, or in excess of, their HIV-negative counterparts[16]. However, high total and saturated fat, as well as low n-3 polyunsaturated fat and fibre intakes, have also been reported in HIV-positive populations, which may indicate overall poor diet quality[16-19]. This suggests that HIV patients may be as affected by the global obesity epidemic as the wider population, and that poor diet quality may be a key factor in the increasing prevalence of overweight and obesity in

HIV-positive populations. The majority of these studies, however, did not include HIV-negative control groups; thereby making the impact of HIV infection itself difficult to ascertain.

In this paper we examined dietary intake and body composition (fat and lean tissue) in ARV-naïve HIV-positive and HIV-negative black urban SA women. In addition, we explored whether the relationships between HIV infection, body composition and dietary variables are influenced by differences in disease severity by comparing affected women with low and relatively preserved $CD_4$ counts.

## Methods

### SUBJECTS

This study was a baseline dietary intake and body composition analysis of HIV-negative and HIV-positive black women participating in a longitudinal assessment of bone health in urban SA. The aim was to recruit 95 (+/-5) HIV-negative and 73 (+/-10) in each of the two HIV-positive groups. This sample size was based on calculations for the longitudinal study to detect a 2% change in lumbar spine BMD, allowing for a between-individual coefficient of variation in BMD of 5%, with 95% confidence and 80% power. Inclusion criteria required women to be over 18 years of age, pre-menopausal, not pregnant or not planning to become pregnant for at least 12 months of the follow-up, and, if HIV positive, not yet using ARVs, and free from any current AIDS-related illness, as defined by WHO or Centers for Disease Control (CDC). 247 HIV-positive (HIV+; n=149) and HIV-negative (HIV-; n=98) women were recruited at Chris Hani Baragwanath Academic Hospital in Soweto, through contact with either the 'ZAZI' voluntary counselling and testing (VCT) centre or the hospital's HIV clinic. Subjects were divided as follows: HIV-negative (group 1; HIV-, n=98), HIV-positive with preserved $CD_4$ counts, not eligible for ARV therapy ($\geq 350 \times 10^6$ cells/l) (group 2; HIV+ non-ARV, n=74) and HIV-positive with low $CD_4$ counts, eligible for ARV therapy ($\leq 200 \times 10^6$ cells/l) due to start ARV (group 3; HIV+ pre-ARV, n=75).

### ANTHROPOMETRY

Weight was measured to the nearest 0.1 kg using a digital scale (Dismed, USA) and height was measured to the nearest 1 mm using a wall-mounted Stadiometer (Holtain, UK) in the Frankfort horizontal plane. These measurements were used to calculate the body mass index (BMI [weight (kg)/height ($m^2$)]) of each individual. Underweight, normal, overweight and obese were defined as BMI <18.5, 18.5-24.9, 25-29.9, ≥30.0 kg/$m^2$, respectively. Waist circumference, taken approximately half-way between the iliac crest and the lowest rib, and hip circumference, taken at the maximum circumference around the hips, were measured using a non-stretchable measuring tape to the nearest 1 cm. All measurements were carried out by trained investigators using standardised procedures and participants wore minimal clothing and no shoes while measurements were taken.



### BODY COMPOSITION

Dual energy X-ray absorptiometry (DXA) scans were performed according to standard procedures using a Hologic QDR 4500A dual-energy X-ray absorptiometer (software version 12.5:7, Hologic Inc., Bedford, MA, USA). Whole body fat and lean mass were analysed as whole body less head (WBLH) because many of the women wore wigs and hair weaves, which could have affected the DXA measurements in the head region. These data have been published by Hamill et al[20]. Trunk and limb (arms and legs) fat masses were derived from DXA scans. Percent fat mass for WBLH, as well as for the trunk and limbs as percentages of WBLH fat mass, were calculated. To compare body fat distribution between the groups, the ratio of trunk: limb fat mass was determined. The ratio of fat mass: lean mass$^2$ was also calculated as this was shown to best describe the relationship between fat and lean mass in this population[20].

### DIETARY INTAKE

The dietary assessment tool used in this study was an interviewer-conducted quantitative food frequency questionnaire (FFQ) developed for use in SA. The questionnaire took on average 40 minutes to complete and included a total of 214 commonly eaten foods. These food items were derived from analyses of 11 dietary surveys conducted in rural and urban SA since 1983, and the list includes all foods eaten by at least 3% of the population[21]. The FFQ was extensively piloted on SA adolescents from the Birth to Twenty cohort at both 15 years (interviewing both adolescents and their primary caregivers, n=150[22]) and 17 years of age (n=1700 (Feeley et al, unpublished results)), as well as on adult research assistants during training, and modified accordingly.

To cater for illiteracy in the SA population, the FFQ utilises food flash cards (high quality photographs) of the food items[23]. Data were collected on the previous week's (7 d) dietary intake, including convenience food products, in order to estimate habitual intake for each participant. Participants were asked to separate the food flash cards into a series of piles: firstly, they went through each food card and created a pile of food items they 'rarely/never' ate or drank. Thereafter, they went through the remaining food cards and created a pile of food items they eat/drink less frequently ('occasional'), and a pile they eat regularly and in the past seven days. The participant was then prompted for information on the frequency and amounts of the regular food items in their

diet consumed, the details of which were recorded on the FFQ. Portion sizes were estimated using household measures and a combination of two-dimensional life-size drawings of foods and utensils, and three-dimensional food models as described and validated by Steyn et al[24]. Items eaten occasionally or rarely/never were also recorded.

Coding involved the conversion of the household measures (for example one cup/one serving spoon/one slice) to grams so that an average intake over the previous seven days could be calculated. Nutrient composition (energy and macronutrients) was estimated, using FoodFinder3, a nutrient analysis software program based on the South African Medical Research Council (MRC) food composition tables[25].

Quality control for dietary data acquisition was undertaken by extensive and repeated training of interviewers, reviewing the questionnaires for missing or spurious data, questioning participants on ambiguous answers and spot-checking questionnaires by a second interviewer (usually the senior nutritionist). The plausibility of the reported energy intake data was assessed according to study specific cut-offs as described by Goldberg et al[26] and Black[27].

United States DRIs[28] for energy and macronutrients were selected for assessing the intakes of the groups compared to recommendations as these are most commonly used in SA and the most useful for comparison with other published data. Nutrient intakes were therefore compared with the Estimated Energy Requirement (EER), the Recommended Dietary Allowance (RDA) for protein and carbohydrate and the Adequate Intake (AI) for fibre in adult women aged 19 to 50 years. The median intakes for carbohydrate, protein and fat as proportions of total energy intake were calculated and compared with the Acceptable Macronutrient Distribution Ranges (AMDR).

Variation in the food items consumed between groups was assessed by comparing the 20 most commonly consumed items, as well as their respective food groups. The top 20 reported food items were ranked from the most to the least consumed according to the mean intake reported in grams per day.

## SOCIO-ECONOMIC STATUS AND EDUCATION

Socio-economic status (SES) was assessed using an asset index similar to that used by McVeigh et al[29]. This scored each participant according to the number of household assets they possessed out of a possible 12 (electricity, television, radio, motor vehicle, fridge, washing machine, telephone, video machine, microwave, MNET television channel, DSTV satellite television, cellular telephone). An asset score percentage was then calculated for each participant ((number of recorded household assets/12) x100).



Level of education was assessed according to the number of years completed at primary, secondary or tertiary level.

## ETHICS

The study was approved by the University of the Witwatersrand Human Research Ethics Committee (HREC Number: M101525) and the Gauteng Department of Health.

## STATISTICAL ANALYSIS

Data were analysed using STATA 11.0 (StataCorp, USA). Where 'inaccurate reporters' were identified, dietary data were truncated using Goldberg cut-offs so that energy and macronutrient intakes represented the lowest or highest plausible intake for under- or over-reporters respectively, according to body size. Continuous variables for subject characteristics, as well as anthropometric and body composition measurements and dietary intake, were not normally distributed and were therefore summarised using the median and interquartile range. Education level and BMI categories were summarised as % in each group. Continuous and categorical variables for subject characteristics were compared between the three study groups using the Kruskal-Wallis test for non-parametric data, and the $Chi^2$ test, respectively. Age was found to be significantly different between groups; therefore all subsequent analyses were adjusted for age. Regression analyses were performed to compare differences in anthropometric and body composition variables between the HIV- group and the HIV+, non-ARV and HIV+, pre-ARV groups combined. Between group comparisons were then made using multiple linear regression models with dummy variables created to distinguish between the HIV+ groups. These methods were repeated for analysis of dietary intake data; however, in addition to age-adjusted analyses, subsequent regression analyses were controlled for both age and total energy intake in order to adjust for the dietary variation attributed to differences in body size between groups. Finally, multiple regression models were used to explore whether any of the variables found to differ between groups were independent predictors of anthropometric and body composition differences. Where appropriate hypotheses were conducted using Bonferonni adjusted alpha levels of 0.016 per test (0.05/3).

## Results

Table 1 summarises the subject characteristics and anthropometric variables for the three study groups (previously described, in part, by Hamill et al (20)). The HIV+ pre-ART group had a significantly lower median $CD_4$ count (175) than the HIV+ non-ARV group (420) as a result of study design. Both SES score and level of education were similar across all groups. The HIV+ pre-ARV group had significantly lower body weight, BMI and waist and hip circumference than the

other groups. The prevalence of overweight and obesity was high in the whole sample; with 59% of subjects being overweight or obese. There was a significant difference in the distribution of subjects across BMI categories between the groups, with the HIV+ pre-ARV group having significantly fewer obese subjects (16%) than both the HIV- (30%; p=0.01) and HIV+ non-ARV (37%; p=0.01) groups. The HIV+ pre-ARV group also had a higher underweight prevalence (11%); approximately 3- and 11-fold higher than the HIV- and HIV+ non-ARV groups, respectively.

There was a significant difference in fat mass between the groups, with the HIV+ pre-ARV group having lower total, trunk and limb fat masses, and body fat percent than the other two groups (Table 2). However, when expressed as a % of whole body fat mass, trunk and limb fat percentages were no longer different between the groups. The HIV+ pre-ARV group had a lower fat/lean$^2$ ratio than the other two groups, however trunk: limb fat mass ratio was not different between the groups.

Table 3 presents the results of multiple regression analyses for BMI , WBLH fat mass and lean mass, trunk fat mass and limb fat mass. Only the variables which contributed significantly to the models are presented. The overall models explain 14% of the variance in both BMI and WBLH fat mass, 34% of the variance in WBLH lean mass and 13% of the variance in both trunk and limb fat masses. Age and being in the HIV+ pre-ARV group significantly contributed to the models for BMI , WBLH fat mass and trunk fat mass, with HIV+ pre-ARV group status being associated with an approximately 4.5 kg/m$^2$ decrease in BMI, an 8 kg decrease in fat mass and a 3.5 kg decrease in trunk fat mass. Age, height and being in the HIV+ pre-ARV group were significant contributors to the variation in both WBLH lean mass and limb fat mass, with an approximately 3 kg lower lean mass and 4 kg lower limb fat mass being associated with HIV+ pre-ARV group status.

The daily energy and macronutrient intakes are presented in Table 4. None of the components of dietary intake were different between HIV- and HIV+ subjects, with the exception of total protein and animal protein intake which were significantly lower in the HIV- than the HIV+ subjects. However, when adjusted for total energy intake, the differences in total and animal protein intake were no longer significant. Between group analyses similarly found no differences between HIV-, HIV+ non-ARV and HIV+ pre-ARV groups in any of the dietary intake variables. Dietary intake exceeded the EER, the RDA for carbohydrate and protein, as well as the AI for fibre across all three study groups.

Carbohydrate, protein, fat and fibre contributed to approximately 54%, 11%, 30% and 4%, respectively of total energy intake across all three groups (data not shown). Intakes, as percentages of total energy intake, were therefore within the AMDR for carbohydrate (45-65%), protein (10-



35%) and fat (20-35%) for all groups. Approximately 8%, 5% and 21% of subjects in the sample had carbohydrate, fat and protein intakes, respectively, below the acceptable range, while 5% and 19% had carbohydrate and fat intakes, respectively, above the acceptable range. Alcohol contributed to less than 1% of total energy intake in all groups.

The 20 most commonly consumed food items and their respective food groups are shown in table 5. Data are presented for the whole sample due to the lack of differences seen in the food items consumed between study groups. The HIV+ pre-ARV group consumed the highest amount of food mass from the top 20 items (1176 g/d) when compared to the HIV- and HIV+ non-ARV groups (929 and 1142 g/d respectively). The most commonly consumed food item was maize meal (made into a stiff porridge/'pap') at a mean intake of 258 g/d. The most commonly recorded food group was cereal and cereal products (featured four times in the top 20); with most cereal products being highly refined 'white carbohydrate'. Fruit and vegetables both featured twice; however the vegetable component included french fries which are high in fat (usually sunflower oil). Sugar consumption was high overall, with granulated white sugar being the most frequently recorded food item in the sample (reported 533 times at an average of 15 g/d) and sweetened carbonated drinks being ranked second with a mean consumption of 197 g/d and equating to approximately 340 kJ of energy per day. Meat and meat products featured three times in the top 20 food items; however the cuts of meat tended to be highly processed and fried in sunflower oil (e.g. polony and battered fried chicken). In addition, fish did not feature in the top 20 food items. Use of fats and oils were very common, with brick/hard margarine and sunflower oil being the regularly reported products (13 g/d and 6 g/d respectively). Condiments, high in fat and/or sugar, were regularly added to meals and snacks, with both atchar, a spicy condiment of mangoes and honey, (19g/d) and tomato sauce (10 g/d) featuring in the top 20 food items consumed.

## Discussion

In this sample of black, urban, SA women, HIV-positive subjects had lower weight and BMIs, as well as lower fat and lean mass and percent body fat than their HIV-negative counterparts. This was primarily as a result of the HIV-positive subjects with low $CD_4$ counts having low measures. Multiple regression analyses showed that HIV+ pre-ARV, but not HIV+ non-ARV, status was a key contributor to differences in WBLH fat mass and WBLH lean mass, as well as both trunk and limb fat masses; associated with 8kg and 3kg less fat and lean mass respectively, as well as 3.5 kg less trunk fat and 4kg less limb fat. This challenges the stereotypical view of HIV as a disease associated with involuntary weight loss and wasting prior to ARV initiation and suggests that weight loss may only become a symptom in this population at more severe disease states.

Although WBLH fat mass, as well as trunk and limb fat masses, were lower in the HIV+ pre-ARV group than the other study groups, there were no differences in relative terms (trunk and limb fat percentages) across all groups. This, together with the similarity found in trunk: limb fat ratio between study groups, suggests lower body fat across all sites rather than an altered fat distribution with advanced HIV infection. This contradicts previous U.S. data which showed an increase in both percent trunk fat and trunk: limb fat ratio and a decrease in peripheral fat independent of ARV treatment in HIV-positive women compared to HIV-negative controls [13]. In addition, it suggests a different pattern of fat loss than that associated with ARV-treatment, where lipodystrophy is characterised by abdominal fat accumulation and subcutaneous fat loss, predominantly at the face, limbs and buttocks [30]. This is speculative, however, and a longitudinal study is currently being undertaken in this population, which will hopefully provide more definitive answers.

As previously documented by Hamill et al [20], the HIV+ pre-ARV group had a lower fat/lean$^2$ ratio than the other two study groups, demonstrating approximately 21% and 16% less fat for each kg of lean mass than the HIV- and HIV+ non-ARV groups, respectively. This provides evidence of lower fat mass, rather than lean tissue, at more advanced stages of HIV infection. Although previous literature has found HIV to be associated with preferential loss of lean compared to fat tissue, particularly in male subjects, a disproportionately higher loss of fat mass has been shown in U.S. women [31]. In addition, a preferential loss of fat rather than lean tissue mass has been demonstrated in males with high body fat percentages at baseline, compared to those with less than 15% body fat [32].

Overweight and obesity were common in the sample overall, with a combined prevalence of 59%, a prevalence higher than that of the national estimate of 55% reported for black women in the 2003 SADHS [4]. Although the distribution of women across BMI cut-offs differed significantly between the HIV+ pre-ARV group and the other two study groups, there was still a 44% prevalence of overweight and obesity in this group, while another 11% were classified as underweight. Even at more advanced stages of HIV infection, women are affected by obesity and this needs to be addressed in the population as a whole.

There were no reported differences in dietary intake across study groups, with the exception of total and animal protein intakes which were lower in the HIV- group than in the HIV+ groups combined. However, when adjusted for total energy intake, these differences were no longer significant. All variation seen for protein intake may therefore have been a result of non-significant differences in total energy consumption rather than real differences in diet quality between groups. These results also highlight that all groups, including the pre-ART group with lower median BMI, consume an obesogenic diet. Given the high levels of inflammation and high carbohydrate intake, these participants are likely to have



a high prevalence of insulin resistance and other metabolic abnormalities. These are areas for potential future research.

Food item and food group analyses showed similar consistency of consumption across the groups, regardless of HIV status. The main contributor to the variation in food mass consumed between the three groups seemed to be pap (maize), which differed by approximately 135 g/d between the HIV+ pre-ARV and the HIV- group and approximately 92 g/d between the HIV pre-ARV group and the HIV+ non-ARV group. Diets in the sample as a whole were very high in refined carbohydrate, which is reflected by the high total digestible carbohydrate intakes (approximately three-fold higher than the RDA of 130 g/d). Consumption of processed and fast food products was common and that of fruits and vegetables rare, with the vegetable items consumed (e.g. tomato and onion stew) usually containing added sugar and/or fat. In addition to added sugar intakes, high sugar-based products were also common; with carbonated soft drinks featuring second in the top 20 food items consumed. This is a concern due to the link seen between sugar-sweetened beverage intake and weight gain, as well as diabetes and cardiovascular disease risk[33;34]. The lack of fish, and therefore n-3 polyunsaturated fatty acids, in the top 20 reported food items should also be addressed due to the important role that these essential fatty acids have in regulating immune function[35]. The highly processed nature of food products, as well as high sugar and fat and low vegetable consumption in the sample is an important health issue and highlights the urgent need to address obesity and its risk factors throughout the population.

Although providing an overall picture of habitual energy and macronutrient intake, as well as of commonly consumed food items, the dietary data had certain limitations. The proportion of women classified as 'under-reporters' was very high (31%, data not shown), suggesting that a substantial number were under-estimating their consumption. This may have been due to the high prevalence of overweight and obesity in the sample, as high BMI has been previously shown to predict underreporting[36]. Due to the negative impact that excluding these participants would have had on sample size, truncation was therefore chosen as the best possible method of minimising the effects of under-reporting on study results. Classification of food items based on the South African FCT was also flawed as it misclassified items such as french fries into the vegetable group, thereby over-estimating vegetable consumption in the sample. This classification should be revised in the future to ensure that vegetables high in starch with added fat are more accurately categorised.

A key limitation of the study was that the participants were not a random sample of the adult female population of Soweto and the surrounding area, as inclusion in the study required the women to present at the Chris Hani Baragwanath Hospitals PHRU, HIV clinic, or VCT centre. Women must, therefore, both know that the services exist and have access to them, must be healthy enough to

present at the hospital, and must have sufficient information on HIV and its risks to be motivated to seek counselling or care. This could mean that the HIV-positive and HIV-negative participants included in the study are not a truly representative sample and may also have better knowledge of poor dietary practices and other health risks than the wider population.

Regardless of these weaknesses, this paper provides unique data on diet and body composition differences between HIV-negative and HIV-positive women at varying levels of immunosuppression prior to treatment. In addition, the longitudinal design of the broader study will allow for future analysis of the changes in anthropometric, body composition and dietary variables in this population at 6 months, 12 months, and 24 months follow up.

In conclusion, our data shows that immunosuppression may be a predictor of anthropometric and body composition changes in HIV-positive women and that, in populations with high obesity prevalence, these differences become evident only at advanced stages of infection. This highlights the need for a change in the way diet and body composition are viewed in HIV-positive patients, while emphasizing that poor dietary habits should be addressed in the female, urban, South African population as a whole.



Reference List

(1) Crum-Cianflone N, Roediger MP, Eberly L, et al. (2010) Increasing rates of obesity among HIV-infected persons during the HIV epidemic. *PLoS One* **5**(4):e10106.

(2) Tate T, Willig AL, Willig JH, et al. HIV infection and obesity: where did all the wasting go? (2012) *Antivir Ther* **17**(7).

(3) UNAIDS. (2010) *UNAIDS Report on the global AIDS epidemic 2009*.

(4) Department of Health, Medical Research Council, OrcMacro. (2007) *South Africa Demographic and Health Survey 2003*. Pretoria, South Africa: Department of Health.

(5) Crum-Cianflone N, Tejidor R, Medina S, et al. (2008) Obesity among patients with HIV: the latest epidemic. *AIDS Patient Care STDS* **22**(12):925-30.

(6) Ferrando SJ, Rabkin JG, Lin SH, et al. (2005) Increase in body cell mass and decrease in wasting are associated with increasing potency of antiretroviral therapy for HIV infection. *AIDS Patient Care STDS* **19**(4):216-23.

(7) Mallon PW, Miller J, Cooper DA, et al. (2003) Prospective evaluation of the effects of antiretroviral therapy on body composition in HIV-1-infected men starting therapy. *AIDS* **17**(7):971-9.

(8) Mwamburi DM, Wilson IB, Jacobson DL, et al. (2005) Understanding the role of HIV load in determining weight change in the era of highly active antiretroviral therapy. *Clin Infect Dis* **40**(1):167-73.

(9) Dave JA, Lambert EV, Badri M, et al. (2011) Effect of nonnucleoside reverse transcriptase inhibitor-based antiretroviral therapy on dysglycemia and insulin sensitivity in South African HIV-infected patients. *J Acquir Immune Defic Syndr* **57**(4):284-9.

(10) Esposito F, Coutsoudis A, Visser J, et al. (2008) Changes in body composition and other anthropometric measures of female subjects on highly active antiretroviral therapy (HAART): A pilot study in KwaZulu-Natal, South Africa. *Southern African Journal of HIV Medicine* **9**(4).

(11) Mercier S, Gueye NF, Cournil A, et al. (2009) Lipodystrophy and metabolic disorders in HIV-1-infected adults on 4- to 9-year antiretroviral therapy in Senegal: a case-control study. *J Acquir Immune Defic Syndr* **1**;51(2):224-30.

(12) Despres JP. (2007) Cardiovascular disease under the influence of excess visceral fat. *Crit Pathw Cardiol* **6**(2):51-9.

(13) Hadigan C, Miller K, Corcoran C, et al. (1999) Fasting hyperinsulinemia and changes in regional body composition in human immunodeficiency virus-infected women. *J Clin Endocrinol Metab* **84**(6):1932-7.

(14) George JA, Venter WD, Van Deventer HE, et al. (2009) A longitudinal study of the changes in body fat and metabolic parameters in a South African population of HIV-positive patients receiving an antiretroviral therapeutic regimen containing stavudine. *AIDS Res Hum Retroviruses* **25**(8):771-81.

(15) Macallan DC, Noble C, Baldwin C, et al. (1995) Energy expenditure and wasting in human immunodeficiency virus infection. *N Engl J Med* **13**;333(2):83-8.

(16) Hattingh Z, Walsh CM, Veldman FJ, et al. (2006) Macronutrient intake of HIV-seropositive women in Mangaung, South Africa. *Nutrition Research* **26**(2):53-8.

(17) Arendt BM, Aghdassi E, Mohammed SS, et al. Dietary intake and physical activity in a Canadian population sample of male patients with HIV infection and metabolic abnormalities. (2008) *Curr HIV Res* **6**(1):82-90.

(18) Duran AC, Almeida LB, Segurado AA, et al. (2008) Diet quality of persons living with HIV/AIDS on highly active antiretroviral therapy. *J Hum Nutr Diet* **21**(4):346-50.

(19) Hendricks KM, Willis K, Houser R, et al. Obesity in HIV infection: dietary correlates. *J Am Coll Nutr* **25**(4):321-31.

(20) Hamill M, Ward K, Pettifor J, et al. Bone mass, body composition and vitamin D status of ARV-naïve, urban, black South African women with HIVinfection, stratified by CD4 count. *Osteoporosis International* (submitted) 2012.

(21) Nel J, Steyn JP. (2002) *Report on South African food consumption studies undertaken among different population groups (1983 - 2000): average intakes of foods most commonly consumed.* Pretoria: Department of Health.



(22) Zingoni C, Norris SA, Griffiths PL et al. (2009) Studying a population undergoing nutrition transition: a practical case study of dietary assessment in urban South African adolescents. *Ecol Food Nutr* **48**(3):178-98.

(23) Steyn N, Senekal M. (2005) *A guide for the use of the dietary assessment and education kit (DAEK).* Cape Town, South Africa: Medical Research Council.

(24) Steyn NP, Senekal M, Norris SA, et al. (2006) How well do adolescents determine portion sizes of foods and beverages? *Asia Pac J Clin Nutr* **15**(1):35-42.

(25) Langenhoven ML, Kruger M, Gouws E, et al. (1991) *MRC Food Composition Tables. 3rd edition.* Parow Vally, Cape Town: Medical Research Council.

(26) Goldberg GR, Black AE, Jebb SA, et al. (1991) Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. *Eur J Clin Nutr* **45**(12):569-81.

(27) Black AE. (2000) Critical evaluation of energy intake using the Goldberg cut-off for energy intake:basal metabolic rate. A practical guide to its calculation, use and limitations. *Int J Obes Relat Metab Disord* **24**(9):1119-30.

(28) Food and Nutrition Board IoM. (2002) *Dietary Reference Intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein, and amino acids.*

(29) McVeigh JA, Norris SA, de Wet T. (2004) The relationship between socio-economic status and physical activity patterns in South African children. *Acta Paediatr* **93**(7):982-8.

(30) James J, Carruthers A, Carruthers J. (2002) HIV-associated facial lipoatrophy. *Dermatol Surg* **28**(11):979-86.

(31) Grinspoon S, Corcoran C, Miller K, et al. (1997) Body composition and endocrine function in women with acquired immunodeficiency syndrome wasting. *J Clin Endocrinol Metab* **82**(5):1332-7.

(32) Mulligan K, Tai VW, Schambelan M. (1997) Cross-sectional and longitudinal evaluation of body composition in men with HIV infection. *J Acquir Immune Defic Syndr Hum Retrovirol* **15**(1):43-8.

(33) Hu FB, Malik VS. (2010) Sugar-sweetened beverages and risk of obesity and type 2 diabetes: epidemiologic evidence. *Physiol Behav* **100**(1):47-54.

(34) Malik VS, Popkin BM, Bray GA, et al. (2010) Sugar-sweetened beverages, obesity, type 2 diabetes mellitus, and cardiovascular disease risk. *Circulation* **121**(11):1356-64.

(35) Calder PC, Yaqoob P. (2009) Omega-3 polyunsaturated fatty acids and human health outcomes. *Biofactors* **35**(3):266-72.

(36) Price GM, Paul AA, Cole TJ, et al. (1997) Characteristics of the low-energy reporters in a longitudinal national dietary survey. *Br J Nutr* **77**(6):833-51.

(37) Wolmarans P, Danster N, Dalton A, et al (eds). (2010) *Condensed food composition tables for South Africa.* Parow Valley, Cape Town: Medical Research Council.



**Table 1. Subject characteristics and anthropometric variables of study groups**

| Variable | HIV- (n=98) (1) | HIV+ non-ARV (n=74) (2) | HIV+ pre-ARV (n=75) (3) | HIV- vs HIV+, P[c] | Between group comparison | P[c] |
|---|---|---|---|---|---|---|
| Age (years) | 28 (23-37) | 33 (29-37 ) | 33 (28-39) | **<0.001**[a] | 1-2 | **<0.001** |
| | | | | | 1-3 | **<0.002** |
| | | | | | 2-3 | 0.92 |
| Current CD4 count x $10^6$ cells/l | ND | 420 (345-472) | 175 (105-226) | NA | 2-3 | **<0.001** |
| **SES [n=217]** | | | | | | |
| SES score (%) | 66.67 (58.33-83.33) | 66.67 (58.33 - 83.33) | 66.67 (50-75) | 0.207[a] | | |
| **Education (%) [n=220]** | | | | | | |
| Primary | 2.4 | 4.1 | 6.1 | | | |
| Secondary | 91.5 | 93.2 | 90.8 | 0.206[b] | | |
| Tertiary | 6.1 | 2.7 | 3.1 | | | |
| **Anthropometry** | | | | | | |
| Weight (kg) | 67.1 (56.8-78.3) | 69.75 (59.6-82.8) | 61.4 (50.9-68.6) | **0.033** | 1-2 | 0.945 |
| | | | | | 1-3 | **<0.001** |
| | | | | | 2-3 | **<0.001** |
| Height (m) | 1.58 (1.54-1.62) | 1.59 ( 1.56-1.62) | 1.59 (1.55-1.63) | **0.015** | 1-2 | **0.029** |
| | | | | | 1-3 | **0.05** |
| | | | | | 2-3 | 0.822 |

| | | | | | | |
|---|---|---|---|---|---|---|
| BMI (kg/m$^2$) | 27.3 (23.1-31.7) | 27.8 (23.3-32.3) | 23.5 (20.4-27) | **<0.002** | 1-2 | 0.479 |
| | | | | | 1-3 | **<0.001** |
| | | | | | 2-3 | **<0.001** |
| Overweight BMI (%) | 35 | 28 | 28 | | | |
| Obese BMI (%) | 30 | 37 | 16 | **<0.01**[b] | | |
| Underweight BMI (%) | 4 | 1 | 11 | | | |
| Waist circumference (cm) | 86 (76-94) | 86 (79-99) | 81 (73-88) | 0.251 | 1-2 | 0.517 |
| | | | | | 1-3 | **0.009** |
| | | | | | 2-3 | **<0.002** |
| Hip circumference (cm) | 106 (97-113) | 106.5 (98-116) | 96.5 (90-106) | **<0.001** | 1-2 | 0.497 |
| | | | | | 1-3 | **<0.001** |
| | | | | | 2-3 | **<0.001** |

**Data are summarised as median (interquartile range) unless otherwise indicated**

[a]**Kruskal-Wallis test, P<0.05**

[b]**Chi$^2$ test, P<0.05**

[c]**Multiple regression adjusted for age, P<0.05**



**Table 2. Body composition variables of study groups**

| DXA [n=245] | HIV- (n=97) (1) | HIV+, non-ARV (n=74) (2) | HIV+, pre-ARV (n=74) (3) | HIV- vs HIV+, P[a] | Between group comparison | P[a] |
|---|---|---|---|---|---|---|
| **WBLH fat mass (kg)** | 24.45 (17.35-30.57) | 25.31 (18.06-32.88) | 18.43 (12.45-25.32) | **0.002** | 1-2 | 0.458 |
| | | | | | 1-3 | **<0.001** |
| | | | | | 2-3 | **<0.001** |
| **WBLH lean mass (kg)** | 37.68 (34.21-41.28) | 39.67 (34.86-42.26) | 36.18 (33.56-39.84) | 0.201 | 1-2 | 0.512 |
| | | | | | 1-3 | **0.005** |
| | | | | | 2-3 | **0.001** |
| **Body fat %** | 39.55 (34.07-43.22) | 39.5 (33.06-44.67) | 32.24 (26.79-41.21) | **<0.001** | 1-2 | 0.372 |
| | | | | | 1-3 | **<0.001** |
| | | | | | 2-3 | **<0.001** |
| **Fat/lean$^2$ (kg/kg$^2$)** | 17.19 (13.76-20.38) | 15.92 (13.36-19.85) | 13.84 (10.15-18.88) | **<0.003** | 1-2 | 0.181 |
| | | | | | 1-3 | **<0.001** |
| | | | | | 2-3 | 0.021[b] |
| **Trunk fat (kg)** | 9.72 (6.68-13.56) | 11.38 (7.39-15.28) | 7.48 (4.64-11.81) | **0.007** | 1-2 | 0.592 |
| | | | | | 1-3 | **<0.001** |
| | | | | | 2-3 | **<0.001** |
| **Trunk fat %** | 41.56 (37.67- 45.65) | 42.85 (38.54-46.38) | 40.92 (35.75-47.07) | 0.717 | 1-2 | 0.602 |
| | | | | | 1-3 | 0.259 |
| | | | | | 2-3 | 0.117 |
| **Limb fat (kg)** | 14 (10.32 - 16.7) | 13.97 ( 10.74-17.93) | 10.75 (7.32-14.15) | **<0.001** | 1-2 | 0.375 |
| | | | | | 1-3 | **<0.001** |
| | | | | | 2-3 | **<0.001** |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Limb fat %** | 58.44 (54.35-62.33) | 57.15 (53.62-61.46) | 59.08 (52.93- 64.25) | 0.717 | 1-2 | 0.602 |
| | | | | | 1-3 | 0.259 |
| | | | | | 2-3 | 0.117 |
| **Trunk: limb fat ratio** | 0.71 (0.6-0.84) | 0.75 (0.63-0.86) | 0.69 (0.56- 0.89) | 0.907 | 1-2 | 0.567 |
| | | | | | 1-3 | 0.445 |
| | | | | | 2-3 | 0.204 |

**Data are summarised as median (interquartile range)**

**[a]Multiple regression adjusted for age, P<0.05**

**[b]Non-significant due to Bonferroni adjustment**



**Table 3. Multiple regression analyses for BMI, WBLH fat mass and WBLH lean mass**

| | β | 95% Confidence Interval | P | | | |
|---|---|---|---|---|---|---|
| **BMI** | | | | | | |
| **Age** | 0.257 | 0.146, 0.367 | **<0.001** | | | |
| **HIV- (ref.)** | 0 | | | $R^2$ | | 0.14 |
| **HIV+, non-ARV** | -0.691 | -2.61, 1.227 | | 0.479 | Adjusted $R^2$ | 0.13 |
| **HIV+, pre-ARV** | -4.487 | -6.394, -2.575 | | **<0.001** | P | **<0.001** |
| **WBLH fat mass** | | | | | | |
| **Age** | 0.347 | 0.166, 0.527 | **<0.001** | | | |
| **HIV- (ref.)** | 0 | | | $R^2$ | | 0.137 |
| **HIV+, non-ARV** | -1.614 | -4.767, 1.539 | | 0.314 | Adjusted $R^2$ | 0.123 |
| **HIV+, pre-ARV** | -7.884 | -11.014, -4.753 | | **<0.001** | P | **<0.001** |
| **WBLH lean mass** | | | | | | |
| **Age** | 0.214 | 0.128, 0.3 | **<0.001** | | | |
| **Height** | 50.826 | 40.207, 61.445 | **<0.001** | | | |
| **HIV- (ref.)** | 0 | | | $R^2$ | | 0.342 |
| **HIV+, non-ARV** | -0.439 | -1.949, 1.07 | | 0.567 | Adjusted $R^2$ | 0.331 |
| **HIV+, pre-ARV** | -3.411 | -4.91, -1.912 | | **<0.001** | P | **<0.001** |
| **Trunk fat** | | | | | | |
| **Age** | 0.208 | 0 .116, 0.3 | **<0.001** | | | |
| **HIV- (ref)** | 0 | | | $R^2$ | | 0.134 |
| **HIV+, non-ARV** | -0.621 | -2.232, 0.99 | | 0.448 | Adjusted $R^2$ | 0.12 |
| **HIV+, pre-ARV** | -3.521 | -5.121, -1.921 | | **<0.001** | P | **<0.001** |
| **Limb fat** | | | | | | |
| **Age** | 0.139 | 0.043, 0.234 | **0.004** | | | |
| **Height** | 12.128 | 0.417, 23.84 | **0.042** | | | |
| **HIV- (ref)** | 0 | | | $R^2$ | | 0.129 |
| **HIV+, non-ARV** | 0.993 | -2.658, 0.672 | | 0.241 | Adjusted $R^2$ | 0.114 |
| **HIV+, pre-ARV** | -4.362 | -6.016, -2.71 | | **<0.001** | P | **<0.001** |

**All variables included in the above models were those which showed significance in prior analyses, P<0.05**

**Table 4. Daily energy and macronutrient intakes of study groups and comparison with dietary recommendations**

| Variable | HIV- (n=98) (1) | HIV+ (n=74) (2) | non-ARV | HIV+ pre-ARV (n=75) (3) | HIV- vs HIV+, P[a] | HIV- vs HIV+, P[b] | Dietary reference intake |
|---|---|---|---|---|---|---|---|
| **Energy (kJ/d)** | 11863 (10179-14404) | 12225 (10541-14767) | | 11773 (9911-16066) | 0.121 | | 10093[b] |
| **Total digestible carbohydrate (g/d)** | 392 (329-459) | 404 (339-503) | | 388 (331-487) | 0.210 | 0.825 | 130[c] |
| **Total fibre (g/d)** | 27 (20-34) | 27 (20-36) | | 26 (20-37) | 0.363 | 0.916 | 25[d] |
| **Total sugars (g/d)** | 80 (56-109) | 86 (56-109) | | 82 (59- 120) | 0.132 | 0.310 | |
| **Total fat (g/d)** | 97 (83-124) | 100 (78-130) | | 88 (71-140) | 0.331 | 0.447 | |
| **Saturated fat (g/d)** | 27 (22-33) | 29 (23-36 ) | | 24 (18-43) | 0.103 | 0.519 | |
| **Polyunsaturated fat (g/d)** | 31 (25-40) | 27 (21-39) | | 28 (22-43) | 0.877 | 0.09 | |
| **Total protein (g/d)** | 81 (65-118) | 84 (71-110) | | 79 (64- 96) | **0.023** | 0.078 | 46[c] |
| **Plant protein (g/d)** | 39 (31-48) | 38 (30-48) | | 40 (32-55) | 0.559 | 0.339 | |
| **Animal protein (g/d)** | 39 (24-48) | 40 (32-54) | | 36 (23-63) | **0.015** | 0.061 | |

**Data are summarised as median (interquartile range)**

**[a]Multiple regression adjusted for age, P<0.05**

**[b]Multiple regression adjusted for age and total energy intake (kJ), P<0.05**

**[b]Estimated Energy Requirement (EER), [c]Recommended Daily Allowance (RDA), [d]Adequate Intake (AI)**

**Table 5. Top 20 food items consumed in the sample**

| | Food item | Food group[a] | Mean g/d |
|---|---|---|---|
| **1** | Maize meal porridge (cooked stiff/pap) | Cereal and cereal products | 258 |
| **2** | Carbonated cold drink (e.g. Coca Cola) | Sugar, syrups and sweets | 197 |
| **3** | Brown bread/rolls | Cereal and cereal products | 121 |
| **4** | Full fat milk | Milk and milk products | 84 |
| **5** | White bread/rolls | Cereal and cereal products | 69 |
| **6** | White rice | Cereal and cereal products | 72 |
| **7** | Banana | Fruit | 53 |
| **8** | Apple | Fruit | 46 |
| **9** | French fries | Vegetables | 34 |
| **10** | Tomato and onion (stewed) | Vegetables | 22 |
| **11** | Chicken, dark meat (fried/roasted) | Meat and meat products | 20 |
| **12** | Atchar (mango) | Sauces, seasonings and flavourings | 19 |
| **13** | Granulated white sugar | Sugar, syrups and sweets | 15 |
| **14** | Brick/hard margarine | Fats and oils | 13 |
| **15** | Tomato sauce | Sauces, seasonings and flavourings | 10 |
| **16** | Batter dipped fried chicken (e.g. KFC) | Meat and meat products | 9 |
| **17** | Cheddar cheese | Milk and milk products | 8 |
| **18** | Polony | Meat and meat products | 7 |
| **19** | Sweets (hard boiled/soft jelly type) | Sugar, syrups and sweets | 6 |
| **20** | Sunflower oil | Fats and oils | 6 |

**[a] Food items grouped according to the current South African MRC Food Composition tables [37]**

Title: Bone mass, body composition and vitamin D status of ARV-naïve, urban, black South African women with HIV infection, stratified by $CD_4$ count.

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Summary:

This is the first report examining vitamin D status and bone mass in African women with HIV infection using DXA with an appropriate HIV-negative control group. Unlike previous publications it demonstrates no difference in BMD or vitamin D status in HIV-positive, at different disease stages, vs. HIV-negative subjects.

Word count: 3210



**Abstract**:

Purpose: Low bone mass and poor vitamin D status have been reported among HIV-positive patients; suggesting HIV or its treatment may increase the risk of osteoporosis, a particular concern for women in countries with high HIV prevalence such as South Africa. We describe bone mass and vitamin D status in urban premenopausal South African women, who were HIV positive but not on ARV therapy.

Methods: Cross-sectional measurement of BMD and body composition by DXA and vitamin D status by serum 25(OH)D concentration. Subjects were recruited into three groups: HIV-negative (n=98) and HIV-positive with preserved $CD_4$ cell count (non-ARV) (n=74) or low $CD_4$ cell counts prior to ARV initiation (pre-ARV) (n=75).

Results: Mean (SD) age of women was 32.1 (7.2) years. Mean $CD_4$ (SD) counts ($x10^6$/L) were 413 (91) and 161 (70) in non-ARV and pre-ARV groups ($p<0.0001$). Pre-ARV women were significantly lighter and had lower mean BMI than the other two groups ($p<0.002$). The pre-ARV group also had significantly less fat and lean mass compared with non-ARV and HIV-negative subjects ($p\leq0.05$). After full adjustment, there were no significant differences in BMD at any site ($p>0.05$) between the groups, nor was vitamin D status significantly different between groups ($p>0.05$); the mean (SD) cohort 25(OH)D being 60 (18) nmol/L.

Conclusions: Contrary to previous studies, these HIV-positive women did not have lower BMD or 25(OH)D concentrations than HIV-negative controls, despite the pre-ARV group being lighter with lower BMI.

**Key words**: Body composition, bone mineral density, Dual energy X-ray absorptiometry, HIV infection, Vitamin D

**Introduction**:

HIV infection and the use of antiretroviral (ARV) medication have been associated with low bone mineral density (BMD) and poor vitamin D status. In a meta-analysis, the prevalence of low BMD in HIV-positive individuals was three times higher than in HIV-negative controls [1-3]. Similarly studies have described high prevalence of low 25-hydroxyvitamin D (25(OH)D) concentrations in HIV-positive patients [4]. Some studies of the effects of HIV and/or its treatment on bone are limited by retrospective design, a preponderance of white, male subjects and lack of HIV-negative controls [5] while others are prospective [6] and do include women [7, 8]. Other studies are limited by confounding by low body weight or other risk factors for low BMD, such as intravenous drug use (IDU), exposure to a large variety of ARV regimes, and measurement of BMD and vitamin D status after varying duration of ARV exposure [6]. The few prospective studies focusing on women have also been limited by some of these aspects [6, 9], and as a result it is difficult to ascertain with certainty if HIV infection and/or its treatment or factors unrelated to HIV infection are contributing factors to the low bone mass and low vitamin D status described in the current literature. In contrast there are data to suggest that after adjusting for body weight BMD is normal or near normal, and that patients on ARV do not have increased rates of bone loss [10, 11]. As a result there is not a definitive consensus on the contribution of HIV infection or ARV exposure on BMD in infected individuals.

In South Africa, estimates of HIV prevalence for 2010 are 10.5% for the total population and 29.3% for women attending antenatal clinics. The epidemic is described as "hyper endemic" because of the high prevalence and continuing drivers of transmission [12-14]. In South Africa individuals generally become eligible for ARV treatment when their $CD_4$ count is less than a nationally-specified threshold. By 2009 56% of those requiring ARV were able to receive them, with the Government intending to increase ARV coverage to 80% by 2011 [12].

Vitamin D has well-known associations with bone health via its role in calcium and phosphate homeostasis, and vitamin D status is considered an important modulator of immune function by some authors [15-17]. In South Africa, adults are largely dependent on the cutaneous synthesis of vitamin D to maintain vitamin D status, as only small amounts of vitamin D are obtained from the diet due to limited food fortification. In Johannesburg (26°S latitude), there is sufficient UVB radiation in sunshine



throughout the year for dermal synthesis of vitamin D [18]. Nevertheless, vitamin D deficiency has been described in tropical/subtropical countries despite the potential for adequate skin exposure to UVB-containing sunshine [19].

The aim of the study presented here was to describe BMD, body composition and vitamin D status in South African women with and without HIV infection, prior to a planned longitudinal study of this cohort to chart the changes in these outcomes over time. We hypothesised that HIV-positive women with low $CD_4$ counts, below the threshold that would make them eligible for ARV treatment, would have lower bone mass, less fat and muscle mass and inferior vitamin D status than HIV-positive women with preserved $CD_4$ counts and HIV-negative women in South Africa.

**Methods:**

**Subjects:**

Urban, black, premenopausal, South African women (n=247) were recruited from clinics in Soweto, Greater Johannesburg, and enrolled into the study between February and July 2010. Subjects were recruited from a Voluntary Counselling and Testing (VCT) clinic and local health clinics. The aim was to recruit 95 HIV-negative and 73 (+/-10) in each of two HIV-positive groups (with or without low CD4 counts). This sample size was based on calculations for the longitudinal study to detect a 2% change in lumbar spine BMD, allowing for a between-individual coefficient of variation in BMD of 5%, with 95% confidence and 80% power. For the study presented here, this sample size was sufficient for a comparison of three groups to allow the detection of mean differences between each pair of groups of around 0.4 SD at 5% significance and 80% power. The study was approved by the University of the Witwatersrand Human Research Ethics Committee (HREC Number: M101525) and the Gauteng Department of Health.

Eligible subjects were adult females (defined as aged greater than 18 years) and premenopausal (defined as regular menses). Other inclusion criteria included a documented negative HIV-test within the last 12 weeks for HIV-negative women and a documented positive HIV-test for all other women. Patient-retained clinic records were scrutinised whenever possible to confirm medical history, current $CD_4$ count, prior exposure to ARVs and concurrent medication use. Exclusion criteria included

conditions associated with abnormal bone metabolism or current use of medication likely to affect bone or vitamin D status such as bisphosphonates. Pregnant and lactating women were excluded as were those with an acute medical condition. The group with the lowest $CD_4$ count were largely recruited after the other groups: May to June and February to April respectively.

Study posters were displayed in the clinic and training sessions undertaken with clinic staff. Women who expressed an interest in the study underwent initial telephone screening, in her language, to ensure inclusion and exclusion criteria were met. Prior to enrolment potential subjects completed a medical and health related questionnaire to assess past and current health status and medication use and to further assess compliance with inclusion and exclusion criteria. Women who remained eligible were enrolled in the full study after they had provided written consent.

The enrolled women consisted of HIV-negative (n=98) and HIV-positive (n=149) subjects. The HIV-positive women were recruited into two prespecified groups: those with relatively preserved $CD_4$ counts (>350 $x10^6$ cells/l), not requiring ARV therapy (non-ARV group) (n=74) and those with low $CD_4$ counts (in the region of 200 $x10^6$ cells/l) requiring ARV initiation (pre-ARV group) (n=75) according to the current SA treatment guidelines [20]. HIV-negative status was confirmed using the Determine™ rapid HIV-antibody test (Alere San Diego, Inc. San Diego, CA ), while HIV-positive status was established using a second platform. HIV-positive women were either newly diagnosed or known to be HIV-positive, but not on ARVs. All HIV-positive women provided an up-to-date (within 3 months) $CD_4$ count prior to enrolling into the study. All HIV-positive women received South African standard of care with respect to ARV provision and clinical follow up. Women requiring urgent ARV initiation were managed in such a way that there would be no delay in ARV initiation if they were to participate in the study.

Women attended the Developmental Pathways for Health Research Unit (DPHRU) facility at the Chris Hani Baragwanath Academic Hospital, after an overnight fast and underwent phlebotomy, anthropometry, and DXA assessment of bone mass and body composition (BC). After phlebotomy, subjects were given breakfast and each received ZAR 50.00 (≈US$6.25) for travel expenses.

**Anthropometry:**



Height was measured to the nearest millimetre using a stadiometer (Holtain, Crosswell, UK). Weight was measured to the last 100 g using a digital scale (Tanita, TBF-410 MA Body Composition Analyzer, Tanita Corporation of America, Inc., Illinois, USA) with participants wearing light clothing and no shoes. BMI was calculated as the participant's weight in kilograms divided by the square of their height in metres ($kg/m^2$). Underweight, normal, overweight and obese were defined as BMI <18.5, 18.5-24.9, 25-29.9, ≥30.0 $kg/m^2$ respectively [21].

**Bone absorptiometry and body composition measurements:**

DXA was performed using an Hologic QDR 4500A dual-energy X-ray absorptiometer (DXA) (Model: Discovery W (S/N 71201) software version 12.5:7 Hologic, Inc., Waltham, MA, USA) according to standard procedures. Scans were conducted using the automatic scan mode, i.e. 'array', 'fast array' or 'slow array', depending on the weight of the subjects. Subjects wore light clothing having removed metal objects, jewellery etc. DXA was used to measure bone mineral content (BMC g), bone area (BA, $cm^2$) and areal BMD ($g/cm^2$), of whole body (WB), total hip (TH), femoral neck (FN) and lumbar spine (LS). The coefficients of variation (CV%) for repeated measurements of the manufacturer's phantom were 0.3%; 0.4% and 0.2% for BA, BMC, and BMD respectively. The CV for repeated measurement by the DXA operator of the LS and TH BMD were 0.7% and 1.0% respectively. DXA scans for WB were analysed using WB less head (WBLH) as many women wore wigs and hair weaves that could not be removed prior to scanning. This artificial hair was of similar density to soft tissue and therefore could cause measurement artefact. Total fat and lean body mass (g) were also measured by DXA.

**Laboratory analysis:**

Blood was collected for 25-hydroxyvitamin D (25(OH)D) analysis, measured by chemi-luminescent immunoassay (Liason) kit (DiaSorin Inc., Stillwater, Minnesota, USA). The blood samples were allowed to clot for a minimum of 20 minutes at room temperature, and the serum was aliquoted and stored at -20°C until analysed. All samples were run in duplicate. The inter-assay CV for low and higher 25(OH)D controls was 10% and 9%, respectively, whereas the intra-assay CV was 8% and 6% respectively. The DPHRU laboratory participates in the International Vitamin D External Quality Assessment Scheme (DEQAS) and holds the certificate of proficiency [22].

**Statistical analysis:**

Data were analysed using DataDesk 6.3.1 (Data Description Inc, Ithaca, NY) and summary statistics were documented as mean (standard deviation (SD)) or median (interquartile range), depending on the distribution. Comparisons were made between the three groups of women using hierarchical linear models; ANOVA (or ANCOVA) and Scheffé post hoc tests were used to compare group means (standard error (SE)). To consider the possible influence of group differences in bone and body size, bone mineral data were adjusted for age, weight, height and bone area, and bone area was adjusted for age, weight and height, using ANCOVA [17]. Preliminary plots of the relationship between fat mass and lean mass in this sample population demonstrated non-linearity. Regression of fat mass on lean mass in the HIV-negative control group with data in natural logarithms gave a power exponent 2.05 ± 0.18 (SE) , indicating that fat mass-to-lean mass$^2$ best described the relationship in this population. The exponent was similar when the data from all three groups were included in the model; 2.07± 0.14. Consequently, a fat mass-to-lean mass$^2$ term was used to describe differences in body composition between the groups, and logarithmic regression was used to adjust fat mass for lean mass in statistical models. BMD SD-scores (SDS) were generated using HIV-negative subjects as the reference population (ref) against which the SDS for each individual HIV-positive woman (i) was derived as follows: [($BMD_i$– mean $BMD_{ref}$ )/$SD_{ref.}$] A $p$ value of ≤0.05 was considered to be statistically significant.

**Results:**

**Subject characteristics (Table 1):**

By design, the mean $CD_4$ count (x $10^6$ cells/l) in the pre-ARV group was significantly lower than that in the non-ARV group (412 (91) and 161 (70) respectively, $p$<0.0001). The mode of acquisition of HIV infection was via heterosexual transmission in all subjects, only one subject reporting IDU in the past.

Mean age (SD) was 32.1(7.2) years with HIV-negative women being significantly but only slightly younger than both groups of HIV-positive women. The age ranges were similar in the three groups (18-49, 22-48 and 19-47 years in HIV-negative, non-ARV and pre-ARV women respectively). Median (IQR) gravidity was 2 (1;3) with both HIV-positive groups having a higher median gravidity compared to the HIV-negative group.



**Anthropometry and body composition (Table 1):**

HIV-negative women tended to be shorter than both groups with HIV infection ($p$=0.06), while HIV positive, pre-ARV women were significantly lighter than the other two groups ($p$<0.05). Median (IQR) BMI of the study cohort was 26.1 (22.4; 31) kg/m$^2$ with BMI in pre-ARV women being significantly lower than in HIV-negative and non-ARV women. Combined overweight and obesity represented 66%, 65% and 44% of subjects in HIV-negative, non-ARV and pre-ARV women respectively , while underweight was present in 4%, 1% and 11% respectively.

There were significant differences in fat mass between groups with pre-ARV women having significantly lower fat mass than non-ARV women ($p$≤0.001). Although lean mass was also lower in pre-ARV compared with non-ARV women ($p$=0.005) the pre-ARV group had lower fat mass to lean mass$^2$ ratio than the other two groups ($p$=0.002). When fully adjusting for lean mass using logarithmic regression, the pre-ARV group had significantly lower fat mass for their lean mass than the other two groups; such that for each unit of lean mass the pre-ARV group (Group 3) had a mean difference (SE) of 21 (5)% less fat than the controls (Group 1), $p$=0.0002, and 16 (5)% less fat than the non-ARV group (Group 2), $p$=0.02.

**Bone measures (Table 2):**

No significant differences in BMD at the TH, FN, LS and WBLH were found, and age and size adjustment did not reveal any differences between groups.. When expressed as SD-scores there were no significant differences between pre-ARV and non-ARV groups in BMD for any site measured ($p$>0.05) and all the mean values were within a -0.5 SD of the HIV-negative reference group. In addition no significant differences were found in BMC values except at WBLH when fully adjusted for age, size and BA (p=0.03). Unadjusted BA was significantly greater in both groups of HIV-positive women than HIV-negative women at some sites but these differences disappeared after adjusting for age and size (see supplementary material for BA and BMC data).

**Vitamin D status (Table 1):**

Mean (SD) 25(OH)D for the whole cohort was 60.1 (18.4) nmol/l and there were no significant differences between groups ($p$>0.05). 25(OH)D concentration was <50 nmol/L in 29.6% of individuals; with similar proportions in each of the groups in this category (26.5%, 29.7% and 33.3% in

HIV-negative, non-ARV and pre-ARV respectively). Very few subjects had a 25(OH)D concentration <25 nmol/L (1.0%, 2.7% and 5.3% in the three groups respectively), despite the slightly greater number of pre-ARV subjects whose blood samples for 25(OH)D measurement were obtained during the winter months.

**Discussion:**

The aim of this study was to determine whether South African HIV-positive women with preserved $CD_4$ counts differed from those with low $CD_4$ counts making them eligible for ARV and to compare each group with HIV-negative women. In this group of urban, South African women, pre-ARV women were significantly lighter than HIV-negative and non-ARV subjects and had lower fat mass than expected for their lean mass, raising the possibility that women with advancing HIV disease preferentially lose fat rather than lean mass. There were no significant differences between groups in BMC or BMD at any site before or after adjustment for age, BA, weight and height and the observed smaller BA in the HIV negative women disappeared after adjustment for age, height and weight. There was no significant difference in vitamin D status between groups with the majority of subjects having a serum concentration >50 nmol/l.

The assessment of 'optimal' vitamin D status is problematic because varying cut offs are used to define sufficiency, insufficiency and deficiency [23]. A concentration below 25 nmol/l is generally recognised as indicating an increased risk of rickets and osteomalacia [24] . The 2010 Institute of Medicine (IOM) report considered that a blood 25(OH)D concentration of 20ng/mL (50nmol/l) to be sufficient for good bone health in "practically all individuals" [25]. However, it noted that evidence was lacking to make a similar statement regarding non-skeletal health. In the context of HIV infection and ARV use, the optimal vitamin D status remains undefined because there may be different requirements for maximal bone health and immune functioning compared with HIV-negative populations. However, in contrast to other reports [4, 26], in our study there were no indications that HIV infection was associated with inferior vitamin D status because there were no significant differences in vitamin D status between the 3 groups, the distributions of 25(OH)D concentration were similar, and vitamin D status appeared to be generally adequate with very few women having a concentration <25 nmol/l.



Contrary to previous reports [9], we found no significant differences in BMD between either group of HIV-positive and HIV-negative women. Full adjustment for bone and body size did not alter these results. This lack of any differences is surprising as HIV-positive women with low $CD_4$ counts, requiring ARV initiation, were significantly lighter, with lower fat and lean mass, than the other women. However, it may reflect the selection criteria for this study because despite recruiting women with low $CD_4$ counts, of clinical concern, women with severe clinical disease received immediate ARV therapy and were thus excluded from the study. It may also be influenced by the fact that the subjects were not intravenous drug users and thus not exposed to the additional effect on BMD that this poses. Another limitation may be that the groups were different in terms of duration of hormonal contraception use, parity and total duration of lactation, however at the time of the study no women were pregnant or lactating. The findings are also limited by the fact that the sample of HIV-positive women was likely to be heterogeneous with respect to immune status and duration of infection. However, most other studies have also recruited HIV-positive subjects in a similar manner and this is unlikely to account for the different findings in our study.

The rates of combined overweight and obesity of 66% and 65% in HIV-negative and non-ARV subjects in this study were greater than the national average in South Africa of 51.5% [27]; even women with advanced HIV-disease (pre-ARV group) had a combined overweight and obesity rate of 44%. It is possible, therefore, that the typically high weight of South African women has a sparing effect on bone in those with HIV infection, even with $CD_4$ counts below the threshold for initiation of ARV intervention.

Historically, overweight has been viewed as protective against osteoporotic fracture, although evidence is emerging that overweight and obesity may be a risk factor for leg fragility fractures in women [28]. In the study population of younger black women in South Africa, there were no significant differences in SD-score, expressed relative to the HIV negative group, according to HIV status at any site. The effects of HIV and its treatment on fracture risk in South Africa are unknown.

The lack of difference between the groups which is at variance from previously reported studies may be the result of true lack of effect of HIV infection or reflect important differences in bone response to HIV between black Africans and Caucasians. The study design in which two distinct groups of HIV-positive women, based on South African eligibility criteria for ARV-treatment plus the inclusion of a

HIV-negative control group strengthens the finding that HIV infection with varying degree of immunosuppression does not appear to be driving alterations in BMD or vitamin D status in these young, urban women. The high rates of overweight may be masking more dramatic differences in BMD and vitamin D in those subjects with advanced clinical HIV-disease not included in this study. Further work is required to address the effects of ARV exposure on bone and vitamin D status as well as the relative effect of 'traditional' osteoporosis risk factors in this population. The data from this study provide an insight into bone health, body composition and vitamin D status in African women living with HIV. They challenge our own hypotheses and previously reported differences in BMD and vitamin D status in HIV-positive subjects living in developed countries and highlight the importance of studying subjects prior to ARV exposure.



There were no conflicts of interest.

**Acknowledgements:**

MMH and AP designed the study.

MMH collected data.

MMH, KAW, JMP, SAN and AP reviewed study design and results.

MMH, KAW and AP analysed data.

All authors contributed to interpretation and the writing of the manuscript.

All authors had full access to the data. AP, JMP and SAN had responsibility for the final decision to submit the manuscript for publication.

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References


1. Brown, T.T. and G.A. McComsey, *Osteopenia and osteoporosis in patients with HIV: a review of current concepts.* Curr Infect Dis Rep, 2006. **8**(2): p. 162-70.
2. Brown, T.T. and R.B. Qaqish, *Antiretroviral therapy and the prevalence of osteopenia and osteoporosis: a meta-analytic review.* Aids, 2006. **20**(17): p. 2165-74.
3. Brown, T.T., et al., *Reduced bone mineral density in human immunodeficiency virus-infected patients and its association with increased central adiposity and postload hyperglycemia.* J Clin Endocrinol Metab, 2004. **89**(3): p. 1200-6.
4. Welz, T., et al., *Efavirenz is associated with severe vitamin D deficiency and increased alkaline phosphatase.* AIDS, 2010. **24**(12): p. 1923-8.
5. Bonjoch, A., et al., *High prevalence of and progression to low bone mineral density in HIV-infected patients: a longitudinal cohort study.* AIDS, 2010. **24**(18): p. 2827-33.
6. Dolan, S.E., J.R. Kanter, and S. Grinspoon, *Longitudinal analysis of bone density in human immunodeficiency virus-infected women.* J Clin Endocrinol Metab, 2006. **91**(8): p. 2938-45.
7. Yin, M., et al., *Bone mass and mineral metabolism in HIV+ postmenopausal women.* Osteoporos Int, 2005. **16**(11): p. 1345-52.
8. Arnsten, J.H., et al., *HIV infection and bone mineral density in middle-aged women.* Clin Infect Dis, 2006. **42**(7): p. 1014-20.
9. Dolan, S.E., et al., *Reduced bone density in HIV-infected women.* AIDS, 2004. **18**(3): p. 475-83.
10. Bolland, M.J., et al., *CLINICAL Review # : low body weight mediates the relationship between HIV infection and low bone mineral density: a meta-analysis.* J Clin Endocrinol Metab, 2007. **92**(12): p. 4522-8.
11. Bolland, M.J., et al., *Bone mineral density remains stable in HAART-treated HIV-infected men over 2 years.* Clin Endocrinol (Oxf), 2007. **67**(2): p. 270-5.
12. PA, M., *Republic of South Africa, Country Progress report on the declaration of commitment on HIV/AIDS, 2010 report*, D.o. Health, Editor. 2010, UNAIDS: UNAIDS. p. 1-126.
13. Africa, S.S., *Mid-year population estimates 2010*. 2010: Pretoria South Africa. p. 1-16.
14. Lehohla, P.J., *Mid-year population estimates 2010*, S.S. Africa, Editor. 2010: Pretoria South Africa. p. 1-16.
15. Adams, J.S., et al., *Vitamin D in defense of the human immune response.* Ann N Y Acad Sci, 2007. **1117**: p. 94-105.
16. Conesa-Botella, A., et al., *Is vitamin D deficiency involved in the immune reconstitution inflammatory syndrome?* AIDS Res Ther, 2009. **6**: p. 4.
17. Liu, P.T., et al., *Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response.* Science, 2006. **311**(5768): p. 1770-3.
18. Pettifor, J.M., F.P. Ross, and L. Solomon, *Seasonal variation in serum 25-hydroxycholecalciferol concentrations in elderly South African patients with fractures of femoral neck.* Br Med J, 1978. **1**(6116): p. 826-7.
19. Schoenmakers, I., G.R. Goldberg, and A. Prentice, *Abundant sunshine and vitamin D deficiency.* Br J Nutr, 2008. **99**(6): p. 1171-3.
20. Motsoaledi, S.A.D.o.H., *CLINICAL GUIDELINES FOR THE MANAGEMENT OF HIV & AIDS IN ADULTS AND ADOLESCENTS*, N.D.o. Health, Editor. 2010.
21. WHO, W.H.O. ***BMI classification***. 2006 [cited 21/1/2013].
22. Poopedi, M.A., S.A. Norris, and J.M. Pettifor, *Factors influencing the vitamin D status of 10-year-old urban South African children.* Public Health Nutr, 2011. **14**(2): p. 334-9.
23. Prentice, A., G.R. Goldberg, and I. Schoenmakers, *Vitamin D across the lifecycle:physiology and biomarkers.* Am J Clin Nutr, 2008. **88**: p. 500S-506S.
24. Nutrition, S.A.C.o., *Update on vitamin D*. 2007, Norwich: TSO (The Stationery Office).
25. Ross, A.C., *Dietary Reference Intakes for Calcium and Vitamin D*, in *IOM*. 2010.





26. Van Den Bout-Van Den Beukel, C.J., et al., *Vitamin D deficiency among HIV type 1-infected individuals in the Netherlands: effects of antiretroviral therapy.* AIDS Res Hum Retroviruses, 2008. **24**(11): p. 1375-82.
27. Kruger, H.S., et al., *Overweight among children decreased, but obesity prevalence remained high among women in South Africa, 1999-2005.* Public Health Nutr, 2011: p. 1-6.
28. Compston, J.E., et al., *Obesity is not protective against fracture in postmenopausal women: GLOW.* Am J Med, 2011. **124**(11): p. 1043-50.

**Table 1. Subject characteristics, anthropometric measurements and vitamin D status as measured by serum 25(OH)D.**

| | Group 1 HIV-negative *n*=98 | Group 2 HIV-positive, non-ARV *n*= 74 | Group 3 HIV-positive, pre-ARV *n*= 75 | Group effect ANOVA *P* |
|---|---|---|---|---|
| Age years | 30.0 (8.1) | 33.5 (6.1) [A] | 33.4 (6.5) [B] | 0.001 |
| HIV status | Negative | Positive | Positive | |
| Current $CD_4$ count x $10^6$ cells/l | ND | 412 (91) | 161 (69) [E] | <0.001 |
| Median (IQR) | | 420 (127;409) | 175 (120;165) | |
| Min | NA | 240 | 18 | |
| Max | NA | 604 | 275 | |
| Gravidity range | 0-5 | 0-6 | 0-6 | |
| Median (IQR) | 1 (0;2) | 2 (2;3) [A] | 2 (1;3) [B] | |
| Current hormonal contraceptive use (%) | 34 (35.4) | 26 (36.6) | 25 (33.3) | 0.9 |
| Current smoking (%) | 10.2 | 13.5 | 8 | 0.2 |
| Height (cm) | 157.6 (5.9) | 159.4(5.9) | 159.2 (5.3) | 0.06 |
| Weight(kg) | 69.7 (17.0) | 72.0 (17.4) | 62.3 (15.2) [C,F] | <0.001 |
| BMI ($kg/m^2$) Median (IQR) | 27.3 (23.1;31.7) | 27.8 (23.3;32.3) | 23.5 (20.5;27.0) [D,F] | |
| Overweight BMI >24.9 $kg/m^2$, <30 $kg/m^2$ (%) | 35 | 28 | 28 | <0.001 |
| Obese BMI >30 $kg/m^2$ (%) | 30 | 37 | 16 | |
| Underweight BMI <18.5 $kg/m^2$ (%) | 4 | 1 | 11 | |
| WBLH Fat (kg) | 26.1 (11.5) | 26.1 (98.3) | 19.7 (92.9) [D,E] | <0.0001 |
| WBLH Lean (kg) | 38.3 (60.8) | 39.5 (62.4) | 36.4 (48.1) [F] | 0.005 |
| Fat /lean$^2$ ($kg/kg^2$) | 17.32 (4.80) | 15.92 (4.56) | 14.58 (5.47) [B,G] | 0.002 |
| 25(OH)D (nmol/L) | 59.7 (16.5) | 59.2 (16.5) | 61.6 (22.3) | 0.7 |
| 25(OH)D (nmol/L) >50, (%) | 73.5 | 70.3 | 66.7 | |
| 25(OH)D (nmol/L) <50, (%) | 26.5 | 29.7 | 33.3 | |
| 25(OH)D (nmol/L) <25, (%) | 1.0 | 2.7 | 5.3 | |



All values are Mean (SD) unless indicated. 25(OH)D, 25 hydroxyvitamin D; ARV, Antiretroviral therapy; cm, centimetres; IQR, interquartile range; kg, kilograms; SD, Standard Deviation. [#] Value multiplied 1000 to illustrate the relative differences in kg.

Letters are used to indicate significance of between-group differences as tested by ANOVA/Scheffé
[A] Significantly different from group 1 $P \leq 0.01$.
[B] Significantly different from group 1 $P \leq 0.01$.
[C] Significantly different from group 1 $P \leq 0.05$.
[D] Significantly different from group 1 $P \leq 0.001$.
[E] Significantly different from group 2 $P \leq 0.001$
[F] Significantly different from group 2 $P \leq 0.01$
[G] Significantly different from group 2 $P \leq 0.05$.

**Table 2. BMD of the three groups of South Africa women**

| | BMD $g/cm^2$ Mean (SD) | | | Group effect* *P* |
|---|---|---|---|---|
| | Group 1 HIV-negative *n*= 98 | Group 2 HIV-positive, non-ARV *n*= 74 | Group 3 HIV-positive, pre-ARV *n*= 75 | |
| Total Hip | 1.013 (0.131) | 0.985 (0.124) | 0.988 (0.125) | 0.3 |
| Femoral Neck | 0.930 (0.114) | 0.916 (0.125) | 0.923 (0.131) | 0.8 |
| Lumbar Spine | 1.018 (0.118) | 1.021 (0.109) | 1.006 (0.128) | 0.7 |
| WBLH | 0.958 (0.079) | 0.943 (0.071) | 0.947 (0.080) | 0.4 |

ARV, Antiretroviral therapy; BMD, bone mineral density ($g/cm^2$); SD, Standard Deviation; WBLH, whole body less head.

*Group effect by ANOVA. There were no significant differences between pairs of groups by Scheffé post hoc tests

**Supplementary data. BMC and BA of the three groups of South African women**

| | Group 1 HIV-negative *n*= 98 Mean (SD) | Group 2 HIV-positive, non-ARV *n*= 74 Mean (SD) | Group 3 HIV-positive, pre-ARV *n*= 75 Mean (SD) | Group effect ANOVA *P* Unadjusted | Adjusted* |
|---|---|---|---|---|---|
| Total Hip | | | | | |
| BMC (g) | 31.4 (5.3) | 31.7 (5.1) | 31.7 (4.8) | 0.8 | 0.1 |
| Area ($cm^2$) | 31.0 (3.08) | 31.7 (3.30) | 32.2 (3.18) [A] | 0.04 | 0.6 |
| Femoral Neck | | | | | |
| BMC (g) | 4.38 (0.59) | 4.38 (0.63) | 4.37 (0.66) | 1.0 | 0.09 |
| Area ($cm^2$) | 4.71 (0.34) | 4.79 (0.33) | 4.76 (0.37) | 0.3 | 1.0 |
| Lumbar Spine | | | | | |
| BMC (g) | 55.0 (9.0) | 57.5 (9.5) | 56.8 (9.4) | 0.2 | 0.9 |
| Area ($cm^2$) | 54.1 (4.84) | 56.1 (5.06) [A] | 56.3 (4.43) [A] | 0.003 | 0.3 |
| WBLH | | | | | |
| BMC(g) | 1606 (232) | 1621 (241) | 1564 (224) | 0.3 | 0.03 |
| Area($cm^2$) | 1670 (145) | 1714 (160) | 1647 (142) [B] | 0.02 | 0.6 |

ARV, Antiretroviral therapy; Area, Bone area ($cm^2$); BMC, bone mineral content (g); SD, Standard Deviation; WBLH, whole body less head.

Letters are used to indicate significance of between-group differences as tested by ANOVA/Scheffé
[A] Unadjusted value significantly different from group 1 $P \leq 0.05$ but not significantly different after size adjustment.
[B] Unadjusted value significantly different from group 2 $P \leq 0.05$ but not significantly different after size adjustment.

*BMC adjusted for age, weight, height and bone area by ANCOVA. BA adjusted for age, weight and height by ANCOVA.





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UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG
Division of the Deputy Registrar (Research)

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
R14/49 Dr Matthew Hamill

CLEARANCE CERTIFICATE — M101025

PROJECT — HIV Vitamin D and Bone Health in South African Women (An investigation into changes in bone mineral density and vitamin D status in black, urban South African HIV positive women and HIV negative controls)

INVESTIGATORS — Dr Matthew Hamill.

DEPARTMENT — Perinatal HIV Research Unit

DATE CONSIDERED — 29/10/2010

DECISION OF THE COMMITTEE* — Approved unconditionally

Unless otherwise specified this ethical clearance is valid for 5 years and may be renewed upon application.

DATE 29/10/2010 CHAIRPERSON ..... (Professor PE Cleaton-Jones)

*Guidelines for written 'informed consent' attached where applicable

cc: Supervisor : Dr Neil Martinson

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DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and ONE COPY returned to the Secretary at Room 10004, 10th Floor, Senate House, University.

I/We fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee. I agree to a completion of a yearly progress report.

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES...

MRC Human Nutrition Research

**Record of SCC Review of LREC/MREC Scientific Protocol**

Title of LREC submission: An investigation into changes in bone mineral density and vitamin D status in black, urban South African HIV positive women and HIV negative controls.

Project Investigator: Dr Matthew Hamill

LREC reference number (obtained from LREC when booking submission in):

**For Completion at SCC**

Date Reviewed by SCC: 4 June 2010

**Outcome of SCC review:** (delete as applicable)
Approved for submission to RGC ~~—~~ N/A
~~Approved for submission to RGC with modifications (see below).~~
~~Not approved for submission to RGC.~~

Comments to be forwarded to PI:

The Director confirms that the MRC will be acting as sponsor for this project: Yes

SCC member who will give PI comments Dr Ann Prentice

Signed at SCC by Director or nominee: Ann Prentice

If applicable, approval by Director or nominee for submission to RGC after modifications

Signed by: not applicable



health and social development
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**GAUTENG DEPARTMENT OF HEALTH AND SOCIAL DEVELOPMENT**

**RESEARCH PROPOSAL EVALUATION FORM**

| | |
|---|---|
| **Researcher Name** | Dr Matthew Hamill / Prof J Pettifor / Associate Prof S. Norris / Dr N. Martinson / Associate Prof A. Karstaedt / Dr A. Prentice |
| **Researcher's contact details** | Matthew.Hamill@mrc-hnr.cam.ac.uk |
| **Institution** | Medical Research Council: Human Nutrition Research |
| **Research Topic** | HIV, vitamin D and bone health in South African women: An investigation into changes in bone mineral density and vitamin D status in black, urban South African HIV positive women and HIV negative controls |
| **Date Received by the Directorate PPR** | 6th June 2011 |
| **Date Received Reviewer** | 30th June 2011 |
| **Final Review Date** | 3rd July 2011 |
| **Date Submitted to Director of PPR** | 4th July 2011 |
| **Research Site(s)** | Volunteers will be recruited from the Perinatal HIV Research Unit, the adult HIV Treatment clinic at Chris Hani Baragwanath Hospital and local community clinics in Soweto. |
| **Type of research** | Prospective cohort study |

| CRITERIA | YES | NO | COMMENTS |
|---|---|---|---|
| 1. Is this research project within the scope of the Department of Health key policy priorities/directives? | X | | |
| 2. Content of Research: | | | |
| • Original work | X | | |
| • New facts, ideas | X | | |
| • Confirmation of uncertain data | X | | |
| • Repetition of known data and consequently of limited importance | | X | |
| • Insufficient research information | | X | |
| • Confusion of topics/questions | | X | |
| 3. Is the title of the research project suitable? | X | | |
| 4. Are the objectives of the research project adequate? | X | | |
| 5. Could the objectives be limited to better focus on the project's main objective? | | X | |
| 6. Writing style | | | |

1

| CRITERIA | YES | NO | COMMENTS |
|---|---|---|---|
| ▪ The text of the proposal is clear | X | | |
| ▪ The nomenclature used is correct | X | | |
| ▪ The references used are relevant, comprehensive and accurate | X | | |
| ▪ The spelling and grammar are correct | X | | |
| ▪ The language needs improvement | | X | |
| ▪ The research proposal needs re-styling and re-writing | | X | |
| 7. Are the research methods appropriate to the study? | X | | |
| 8. Is data collection method in line with the study design? | X | | |
| 9. Does the study have ethical approval? If yes, name the ethics committee. | X | | Wits: M101025 |
| 10. Is the definition and measurement of variables consistent with the scope of the proposal? | X | | |
| 11. Is the time frame of the proposal adequate to meet the objectives? | X | | |
| 12. Is the method of dissemination of the results of the research project stated? | | X | It is recommended that the researchers specify how the results will be shared / disseminated |
| 13. Is any possible conflict of interests clarified? | n/a | | |
| 14. Are financial implications and financial support transparent? | x | | |

**SUMMARY OF PROPOSAL**

This study will determine the prevalence of and examine factors associated with low bone mineral density (BMD) and vitamin D status in urban black South African women. It will measure baseline BMD, 25-hydroxy vitamin D (25 OHD) and biochemical markers of bone turnover in 2 groups of HIV+ women; those commencing antiretroviral therapy (ART) and those not requiring ART. These will be compared with data collected from an HIV negative control group. Measurements will be repeated at 12 months. Lifestyle, dietary and physical activity data will also be collected.

The initial evaluation will be over 12 months (for the purpose of Matthew Hamill's PhD programme); volunteers will be invited for further visits at 2 and 3 years.

The research is a collaboration between the following partners:

1. Dr Hammill: conducting PhD research with the Nutrition and Bone Health programme based at MRC Human Nutrition Research (HNR), Cambridge UK;
2. The South African MRC/University of the Witwatersrand Mineral Metabolism Research Unit (MMRU) and the University of the Witwatersrand's Birth to Twenty (Bt20) cohort (Professor John Pettifor and Dr Shane Norris)
3. Perinatal HIV Research Unit (PHRU) (Dr Neil Martinson) for expertise on HIV-infection.

**Background**

HIV and osteoporosis have been viewed for decades as affecting disparate sections of the world's population, with HIV typically seen as a disease of the young and those of reproductive age in Africa and osteoporosis a disease of the elderly in the west. However this perception is changing. In the last decade there has been a steady increase in interest in HIV



associated bone disease with an emphasis on osteoporosis and osteopenia. The advent of highly active anti HIV therapy (ART) has dramatically changed the prognosis of HIV disease and seen a switch in emphasis from infection to non-communicable related morbidity. As a result there is a merging of these 2 major global health problems.

Early studies found no relationship between HIV and loss of bone , but since 2000 there have been numerous reports of low bone mineral density (BMD) and HIV disease. In recent years there has been a growing interest in the association between HIV infection and reduced vitamin D concentrations with skeletal and non-skeletal ramifications. Most data describing reduced BMD and vitamin D in the context of HIV are cross sectional or retrospective and come predominantly from white, male populations in North America and Europe. The burden of HIV lies squarely in Sub Saharan Africa and it is in areas of high HIV prevalence that long term skeletal sequelae are likely to be of greatest importance. Furthermore women are traditionally at greatest risk of fragility fractures making a study of black African women even more pertinent.

To date there is conflicting evidence about the prevalence of bone disease in HIV infected individuals. The global costs of osteoporosis are enormous, and it is also estimated that fracture risk is likely to be particularly prominent in the developing world with its adoption of a more westernised lifestyle. There is a paucity of data on Sub Saharan African osteoporosis and associated costs. Estimates for global costs of HIV are also enormous. Combined, these two conditions, particularly if synergistic, have the potential to pose a huge disease burden to rapidly developing countries such as South Africa that have growing communicable and non communicable disease epidemics such as diabetes and HIV. It is possible that South Africa will, in the future, develop an epidemic of fragility fractures to mirror its HIV epidemic. It is therefore possible that there will be overlapping epidemics of HIV infection and osteoporosis in aging populations in SSA.

**Objectives**

The study will investigate whether HIV associated bone loss and low 25OHD are prevalent in black, urban South African women and allow quantification of the magnitude of any effects and their clinical relevance.

**Nature of the study**

This is a prospective cohort study. It will use subjects who are patients (HIV +) and controls (HIV-) however it is not a clinical trial. There will be no intervention component to this study. The study design has 2 distinct elements; the first is a 12 month follow up period that will allow for data analysis and hypothesis testing within the time constraints of the PhD project. Secondly the study design will allow for subjects to be followed up at 2 and 3 years post enrolment to assess changes in bone markers, BMD and vitamin D status. This aspect of the design will allow more longitudinal analysis of the effects of HIV infection and ART on bone outcomes.

**METHODS**

***Sample size***

217 subjects will be recruited, consisting of 85 HIV-negative control women and 66 in each HIV-positive group (i.e. 132) Cases will be split 50:50 into those who start on ART (Group 1a) and those who will be anticipated to remain ART naïve through the duration of follow up (Group 1b). To account for potential drop outs 146 cases and 95 controls (n=241) will be recruited.

Participants will be Black Urban South African adult, premenopausal women from Soweto, Johannesburg and the surrounding areas. Volunteers will be approached from various sources. These will include the Perinatal HIV research unit, the adult HIV Treatment clinic at Chris Hani Baragwanath Hospital and local community clinics in Soweto. Health workers and potential subjects will be informed that the study will recruit both HIV-negative and HIV-positive women.

***Study design***

All subjects will be assessed for eligibility and informed consent taken before enrolment. Enrolment will be considered complete when the subject undergoes and completes baseline evaluation (i.e. blood sampling, urine sampling, anthropometry measurement, BP, grip strength, DXA measurement for bone and body composition). After the baseline visit all subjects will be contacted monthly via telephone (or home visit if unavailable on the telephone) to undertake a morbidity questionnaire. A mid study visit will take place at 6 months post baseline (blood sampling, dietary evaluation, DXA measurement etc), and the final study visit for the first stage of the study will take place at 12 months post baseline, when they will be tested again. All subjects will be invited to take part in a 2 year and 3 year (post enrolment) follow up.

Volunteers will be asked to attend a further visit at 2 years post baseline, and at this visit will be asked to attend a further visit at 3 years (36 months) post baseline.

**REVIEWER'S FINAL CONCLUSION**

The proposed study will contribute towards a better understanding of the possible future challenges associated with having a large proportion of the population on ART and is recommended for approval.

**Reviewed by**

Date: 4/07/2011

**Recommended / not recommended**

S. le Roux, Director PPR
Date: 4/07/2011

**Recommended / not recommended**

Dr A. Rahman, Chief of Operations
Date: 07/07/2011

**Approved / not approved**

Dr K.S. Chetty, HOD for Health
Date: 11/07/2011





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*Public Health Nutrition: page 1 of 11*

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# Dietary intake and body composition in HIV-positive and -negative South African women

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## Abstract

*Objective:* The present paper examines dietary intake and body composition in antiretroviral (ARV)-naïve HIV-positive compared with HIV-negative South African women, as well as the impact of disease severity on these variables.

*Design:* Baseline data from a longitudinal study assessing bone health in HIV-negative and HIV-positive premenopausal South African women over 18 years of age were used. Anthropometry and body composition, measured by dual energy X-ray absorptiometry, were analysed together with dietary intake data assessed using an interviewer-based quantitative FFQ.

*Setting:* Soweto, Johannesburg, South Africa.

*Subjects:* Black, urban South African women were divided into three groups: (i) HIV-negative ($HIV^-$; *n* 98); (ii) HIV-positive with preserved CD4 counts ($HIV^+$ non-ARV; *n* 74); and (iii) HIV-positive with low CD4 counts and due to start ARV treatment ($HIV^+$ pre-ARV; *n* 75).

*Results:* The prevalence of overweight and obesity was high in this population (59 %). The $HIV^+$ pre-ARV group was lighter and had a lower BMI than the other two groups (all $P < 0{\cdot}001$). $HIV^+$ pre-ARV women also had lower fat and lean masses and percentage body fat than their $HIV^-$ and $HIV^+$ non-ARV counterparts. After adjustment, there were no differences in macronutrient intakes across study groups; however, fat and sugar intakes were high and consumption of predominantly refined food items was common overall.

*Conclusion:* HIV-associated immunosuppression may be a key determinant of body composition in HIV-positive women. However, in populations with high obesity prevalence, these differences become evident only at advanced stages of infection.

**Keywords**
HIV
Diet
Body composition
Obesity

The introduction of antiretroviral (ARV) therapy has dramatically altered the morbidity profile of HIV-positive populations, with an increase in prevalence of non-communicable disease risk factors such as overweight and obesity being observed[1,2]. This is of particular concern in South Africa where, among adults over 15 years of age, 17·8 % were estimated to be living with HIV in 2008[3] and approximately 45 % were found to be overweight or obese in the 2003 Demographic and Health Survey (SADHS)[4].

Although the public health impact of HIV and associated non-communicable disease risk is greatest in low- to middle-income countries, to date most studies have been conducted in high-income countries, on predominantly ARV-treated males with low BMI[5–8]. Lipodystrophy, a commonly recognized side-effect of certain ARV drugs, has been linked to visceral fat accumulation and metabolic disorders such as dyslipidaemia and glucose intolerance[9]. In black South African (SA) women, ARV-associated increases in BMI, fat mass, percentage body fat and waist circumference, but not in lean body mass or waist:hip ratio, have been shown[10]. This increase in fat, as well as the proposed tendency towards visceral rather than subcutaneous fat accumulation[11], must therefore be further explored in this population due to the potential long-term negative effects on disease risk and health[12].

While the evidence for body composition changes associated with ARV drugs continues to grow, there is less research focusing on the effect of the HIV infection itself. Hadigan *et al.*[13] found that, independent of ARV, HIV-positive women in the USA demonstrated a higher percentage body fat and truncal fat and lower percentage lean body mass than HIV-negative controls. In addition, other data suggest that HIV-positive patients with higher

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Public Health Nutrition

BMI, skinfold thicknesses and insulin concentrations prior to ARV initiation may be more likely to develop lipodystrophy over 2 years of ARV drug use[14].

Although HIV-positive status has traditionally been associated with low energy intake, most likely as a result of reduced appetite[15], SA data show energy intake in HIV-positive individuals to be equivalent to, or in excess of, that of their HIV-negative counterparts[16]. However, high total and saturated fat intakes, as well as low *n*-3 PUFA and fibre intakes, have also been reported in HIV-positive populations, which may indicate overall poor diet quality[16–19]. This suggests that HIV patients may be as affected by the global obesity epidemic as the wider population, and that poor diet quality may be a key factor in the increasing prevalence of overweight and obesity in HIV-positive populations. The majority of these studies, however, did not include HIV-negative control groups; thereby making the impact of HIV infection itself difficult to ascertain.

In the present paper we examine dietary intake and body composition (fat and lean tissue) in ARV-naïve HIV-positive and HIV-negative black urban SA women. In addition, we explore whether the relationships between HIV infection, body composition and dietary variables are influenced by differences in disease severity by comparing affected women with low and relatively preserved CD4 counts.

## Methods

### *Participants*

The present study was a baseline dietary intake and body composition analysis of HIV-negative and HIV-positive black women participating in a longitudinal assessment of bone health in urban South Africa. The aim was to recruit ninety-five (±5) HIV-negative and seventy-three (±10) in each of the two HIV-positive groups. This sample size was based on calculations for the longitudinal study to detect a 2 % change in lumbar spine bone mineral density, allowing for a between-individual CV in bone mineral density of 5 %, with 95 % confidence and 80 % power. Inclusion criteria required women to be over 18 years of age; premenopausal; not pregnant or not planning to become pregnant for at least 12 months of the follow-up; and, if HIV positive, not yet using ARV and free from any current AIDS-related illness as defined by the WHO or the US Centers for Disease Control and Prevention. Two hundred and forty-seven women in total, HIV-positive ($HIV^+$; *n* 149) and HIV-negative ($HIV^-$; *n* 98), were recruited at Chris Hani Baragwanath Academic Hospital in Soweto, through contact with either the 'ZAZI' voluntary counselling and testing centre or the hospital's HIV clinic. Participants were divided as follows: (i) HIV-negative (group 1; $HIV^-$, *n* 98); (ii) HIV-positive with preserved CD4 counts ($\geq 350 \times 10^6$ cells/l), not eligible for ARV therapy (group 2; $HIV^+$ non-ARV, *n* 74); and (iii) HIV-positive with low CD4 counts ($\leq 200 \times 10^6$ cells/l), eligible for ARV therapy and due to start ARV (group 3; $HIV^+$ pre-ARV, *n* 75).

### *Anthropometry*

Weight was measured to the nearest 0·1 kg using a digital scale (Scales 2000, Durban, South Africa) and height was measured to the nearest 1 mm using a wall-mounted stadiometer (Holtain, Crymych, UK) and with the participant's head in the Frankfort horizontal plane. These measurements were used to calculate the BMI (weight (kg) divided by the square of height ($m^2$)) of each participant. Underweight, normal weight, overweight and obese were defined as BMI $< 18{\cdot}5\ kg/m^2$, BMI $= 18{\cdot}5$–$24{\cdot}9\ kg/m^2$, BMI $= 25{\cdot}0$–$29{\cdot}9\ kg/m^2$ and BMI $\geq 30{\cdot}0\ kg/m^2$, respectively. Waist circumference, taken approximately half-way between the iliac crest and the lowest rib, and hip circumference, taken at the maximum circumference around the hips, were measured using a non-stretchable measuring tape to the nearest 1 cm. All measurements were carried out by trained investigators using standardised procedures and participants wore minimal clothing and no shoes while measurements were taken.

### *Body composition*

Dual energy X-ray absorptiometry (DXA) scans were performed according to standard procedures using a Hologic QDR 4500A dual-energy X-ray absorptiometer (software version 12·5:7; Hologic Inc., Bedford, MA, USA). Whole-body fat and lean mass were analysed as whole body less head (WBLH) because many of the women wore wigs and hair weaves, which could have affected the DXA measurements in the head region. These data have been published by Hamill *et al.*[20]. Trunk and limb (arms and legs) fat masses were derived from DXA scans. Percentage fat mass for WBLH, as well as for the trunk and limbs as percentages of WBLH fat mass, were calculated. To compare body fat distribution between the groups, trunk:limb fat mass was determined. Fat mass:lean mass$^2$ was also calculated as this was shown to best describe the relationship between fat and lean mass in this population[20].

### *Dietary intake*

The dietary assessment tool used in the present study was an interviewer-conducted quantitative FFQ developed for use in South Africa. The questionnaire took on average 40 min to complete and included a total of 214 commonly eaten foods. These food items were derived from analyses of eleven dietary surveys conducted in rural and urban South Africa since 1983, and the list includes all foods eaten by at least 3 % of the population[21]. The FFQ was extensively piloted on SA adolescents from the Birth to Twenty cohort at both 15 years (interviewing both adolescents and their primary caregivers, *n* 150[22]) and 17 years of age (*n* 1700 (AB Feeley, unpublished results)), as well as on adult research assistants during training, and modified accordingly.

To cater for illiteracy in the SA population, the FFQ utilises food flash cards (high-quality photographs) of the food items[23]. Data were collected on the previous week's (7 d) dietary intake, including convenience food products, in order to estimate habitual intake for each participant. Participants were asked to separate the food flash cards into a series of piles. First, they went through each food card and created a pile of food items they 'rarely/never' eat or drink. Thereafter, they went through the remaining food cards and created a pile of food items they eat/drink less frequently ('occasional'), and a pile they eat regularly and in the past 7 d. The participant was then prompted for information on the frequency and amounts of the food items consumed regularly in her diet, the details of which were recorded on the FFQ. Portion sizes were estimated using household measures and a combination of two-dimensional life-size drawings of foods and utensils and three-dimensional food models as described and validated by Steyn *et al.*[24]. Items eaten occasionally or rarely/never were also recorded.

Coding involved the conversion of the household measures (e.g. one cup/one serving spoon/one slice) to grams so that an average intake over the previous 7 d could be calculated. Nutrient composition (energy and macronutrients) was estimated using FoodFinder3, a nutrient analysis software program based on the SA Medical Research Council food composition tables[25].

Quality control for dietary data acquisition was undertaken by extensive and repeated training of interviewers, reviewing the questionnaires for missing or spurious data, questioning participants on ambiguous answers and spot-checking questionnaires by a second interviewer (usually the senior nutritionist). The plausibility of the reported energy intake data was assessed according to study-specific cut-offs as described by Goldberg *et al.*[26] and Black[27].

The US Dietary Reference Intakes[28] for energy and macronutrients were selected for assessing the intakes of the study groups compared with recommendations, as these are most commonly used in South Africa and the most useful for comparison with other published data. Nutrient intakes were therefore compared with the Estimated Energy Requirement, the RDA for protein and carbohydrate and the Adequate Intake for fibre in adult women aged 19–50 years. The median intakes for carbohydrate, protein and fat as proportions of total energy intake were calculated and compared with the Acceptable Macronutrient Distribution Ranges.

Variation in the food items consumed between study groups was assessed by comparing the twenty most commonly consumed items, as well as their respective food groups. The top twenty reported food items were ranked from the most to the least consumed according to the mean intake reported in grams per day.

### *Socio-economic status and education*

Socio-economic status (SES) was assessed using an asset index similar to that used by McVeigh *et al.*[29]. This scored each participant according to the number of household assets she possessed out of a possible twelve (electricity, television, radio, motor vehicle, fridge, washing machine, telephone, video machine, microwave, MNET television channel, DSTV satellite television, cellular telephone). An asset score percentage was then calculated for each participant ((number of recorded household assets/12) × 100).

Level of education was assessed according to the number of years completed at primary, secondary or tertiary level.

### *Ethics*

The study was approved by the University of the Witwatersrand Human Research Ethics Committee (HREC Number: M101525) and the Gauteng Department of Health. Individuals gave written consent prior to enrolment into the study.

### *Statistical analysis*

Data were analysed using the statistical software package STATA 11·0. Where 'inaccurate reporters' were identified, dietary data were truncated using Goldberg cut-offs so that energy and macronutrient intakes represented the lowest or highest plausible intake for under- or over-reporters, respectively, according to body size. Continuous variables for participant characteristics, as well as anthropometric and body composition measurements and dietary intake, were not normally distributed and were therefore summarised using the median and interquartile range. Education level and BMI categories were summarised as percentage in each group. Continuous and categorical variables for participant characteristics were compared between the three study groups using the Kruskal–Wallis test for non-parametric data and the $\chi^2$ test, respectively. Age was found to be significantly different between groups; therefore all subsequent analyses were adjusted for age. Regression analyses were performed to compare differences in anthropometric and body composition variables between the $HIV^-$ group and the $HIV^+$ non-ARV and $HIV^+$ pre-ARV groups combined. Between-group comparisons were then made using multiple linear regression models with dummy variables created to distinguish between the $HIV^+$ groups. These methods were repeated for analysis of dietary intake data; however, in addition to age-adjusted analyses, subsequent regression analyses were controlled for both age and total energy intake in order to adjust for the dietary variation attributed to differences in body size between groups. Finally, multiple regression models were used to explore whether any of the variables found to differ between groups were independent predictors of anthropometric and body composition



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differences. Where appropriate, hypotheses were conducted using Bonferonni-adjusted $\alpha$ levels of 0·016 per test (0·05/3).

## Results

Table 1 summarises the participant characteristics and anthropometric variables for the three study groups (previously described, in part, by Hamill *et al.*[20]). The $HIV^+$ pre-ARV group had a significantly lower median CD4 count ($175 \times 10^6$ cells/l) than the $HIV^+$ non-ARV group ($420 \times 10^6$ cells/l) as a result of the study design ($P < 0·001$). Both SES score and level of education were similar across all groups. The $HIV^+$ pre-ARV group had significantly lower body weight, BMI and waist and hip circumference than the other groups (most $P < 0·001$). The prevalence of overweight and obesity was high in the whole sample, with 59 % of women being overweight or obese. There was a significant difference in the distribution of participants across BMI categories between the groups, with the $HIV^+$ pre-ARV group having significantly fewer obese individuals (16 %) than both the $HIV^-$ (30 %; $P = 0·01$) and $HIV^+$ non-ARV (37 %; $P = 0·01$) groups. The $HIV^+$ pre-ARV group also had a higher underweight prevalence (11 %; $P < 0·01$), approximately three- and eleven-fold higher than the $HIV^-$ and $HIV^+$ non-ARV groups, respectively.

There was a significant difference in fat mass between the groups, with the $HIV^+$ pre-ARV group having lower total ($P < 0·001$), trunk ($P < 0·001$) and limb fat masses ($P < 0·001$), and percentage body fat ($P < 0·001$), than the other two groups (Table 2). However, when expressed as a percentage of whole-body fat mass, trunk and limb fat percentages were no longer different between the groups. The $HIV^+$ pre-ARV group had lower fat mass:lean mass$^2$ than the other two groups; however, trunk:limb fat mass was not different between the groups. Correlations confirmed the relationship between CD4 count and fat mass ($r = 0·273$; $P = 0·019$) and CD4 count and lean mass ($r = 0·299$; $P = 0·05$).

Table 3 presents the results of multiple regression analyses for BMI, WBLH fat mass and lean mass, trunk fat mass and limb fat mass. Only the variables which contributed significantly to the models are presented. The overall models explain 14 % of the variance in both BMI and WBLH fat mass, 34 % of the variance in WBLH lean mass and 13 % of the variance in both trunk and limb fat masses. Age and being in the $HIV^+$ pre-ARV group significantly contributed to the models for BMI, WBLH fat mass and trunk fat mass (all $P < 0·001$), with $HIV^+$ pre-ARV group status being associated with an approximately 4·5 kg/m$^2$ decrease in BMI, an 8 kg decrease in WBLH fat mass and a 3·5 kg decrease in trunk fat mass. Age, height and being in the $HIV^+$ pre-ARV group were significant contributors to the variation in both WBLH lean mass (all $P < 0·001$) and limb fat mass ($P = 0·004$, $P = 0·042$ and $P < 0·001$, respectively), with an approximately 3 kg lower lean mass and 4 kg lower limb fat mass being associated with $HIV^+$ pre-ARV group status.

The daily energy and macronutrient intakes are presented in Table 4. None of the components of dietary intake were different between $HIV^-$ and $HIV^+$ participants, with the exception of total protein and animal protein intakes which were significantly lower in the $HIV^-$ than the $HIV^+$ individuals ($P = 0·023$ and $P = 0·015$, respectively). However, when adjusted for total energy intake, the differences in total and animal protein intake were no longer significant. Between-group analyses similarly found no differences between $HIV^-$, $HIV^+$ non-ARV and $HIV^+$ pre-ARV groups in any of the dietary intake variables. Dietary intake exceeded the Estimated Energy Requirement, the RDA for carbohydrate and protein, as well as the Adequate Intake for fibre across all three study groups.

Carbohydrate, protein, fat and fibre accounted for approximately 54 %, 11 %, 30 % and 4 %, respectively, of total energy intake across all three groups (data not shown). Intakes, as percentages of total energy intake, were therefore within the Acceptable Macronutrient Distribution Ranges for carbohydrate (45–65 %), protein (10–35 %) and fat (20–35 %) for all groups. Approximately 8 %, 5 % and 21 % of participants in the sample had carbohydrate, fat and protein intakes, respectively, below the acceptable range, while 5 % and 19 % had carbohydrate and fat intakes, respectively, above the acceptable range. Alcohol accounted for less than 1 % of total energy intake in all groups.

The twenty most commonly consumed food items and their respective food groups are shown in Table 5. Data are presented for the whole sample due to the lack of differences seen in the food items consumed between study groups. The $HIV^+$ pre-ARV group consumed the highest amount of food mass from the top twenty items (1176 g/d) compared with the $HIV^-$ and $HIV^+$ non-ARV groups (929 and 1142 g/d, respectively). The most commonly consumed food item was maize meal (made into a stiff porridge/'pap') at a mean intake of 258 g/d. The most commonly recorded food group was cereal and cereal products (featured four times in the top twenty), with most cereal products being highly refined 'white carbohydrate'. Fruit and vegetables both featured twice; however, the vegetable component included French fries which are high in fat (usually sunflower oil). Sugar consumption was high overall, with granulated white sugar being the most frequently recorded food item in the sample (reported 533 times at an average of 15 g/d) and sweetened carbonated drinks being ranked second with a mean consumption of 197 g/d and equating to approximately 340 kJ of energy daily. Meat and meat products featured three times in the top twenty food items; however, the cuts of meat tended to be highly processed and fried in sunflower oil (e.g. polony and



**Table 1** Participant characteristics and anthropometric variables according to study group: premenopausal black women, Soweto, Johannesburg, South Africa

| Variable | $HIV^-$ ($n$ 98) (1) | | $HIV^+$ non-ARV ($n$ 74) (2) | | $HIV^+$ pre-ARV ($n$ 75) (3) | | | Between-group comparison | |
|---|---|---|---|---|---|---|---|---|---|
| | Median | IQR | Median | IQR | Median | IQR | $P$*, $HIV^-$ $v.$ $HIV^+$ | | $P$* |
| Age (years) | 28 | 23–37 | 33 | 29–37 | 33 | 28–39 | <0·001† | (1) $v.$ (2) | <0·001 |
| | | | | | | | | (1) $v.$ (3) | <0·002 |
| | | | | | | | | (2) $v.$ (3) | 0·92 |
| Current CD4 count ($\times 10^6$ cells/l) | ND | – | 420 | 345–472 | 175 | 105–226 | NA | (2) $v.$ (3) | <0·001 |
| SES ($n$ 217) | | | | | | | | | |
| SES score (%) | 66·67 | 58·33–83·33 | 66·67 | 58·33–83·33 | 66·67 | 50·00–75·00 | 0·207† | | |
| Education (%) ($n$ 220) | | | | | | | | | |
| Primary | 2·4 | – | 4·1 | – | 6·1 | – | | | |
| Secondary | 91·5 | – | 93·2 | – | 90·8 | – | 0·206‡ | | |
| Tertiary | 6·1 | – | 2·7 | – | 3·1 | – | | | |
| Anthropometry | | | | | | | | | |
| Weight (kg) | 67·1 | 56·8–78·3 | 69·75 | 59·6–82·8 | 61·4 | 50·9–68·6 | 0·033 | (1) $v.$ (2) | 0·945 |
| | | | | | | | | (1) $v.$ (3) | <0·001 |
| | | | | | | | | (2) $v.$ (3) | <0·001 |
| Height (m) | 1·58 | 1·54–1·62 | 1·59 | 1·56–1·62 | 1·59 | 1·55–1·63 | 0·015 | (1) $v.$ (2) | 0·029 |
| | | | | | | | | (1) $v.$ (3) | 0·05 |
| | | | | | | | | (2) $v.$ (3) | 0·822 |
| BMI (kg/m$^2$) | 27·3 | 23·1–31·7 | 27·8 | 23·3–32·3 | 23·5 | 20·4–27·0 | <0·002 | (1) $v.$ (2) | 0·479 |
| | | | | | | | | (1) $v.$ (3) | <0·001 |
| | | | | | | | | (2) $v.$ (3) | <0·001 |
| Overweight (%) | 35 | – | 28 | – | 28 | – | | | |
| Obese (%) | 30 | – | 37 | – | 16 | – | <0·01‡ | | |
| Underweight (%) | 4 | – | 1 | – | 11 | – | | | |
| Waist circumference (cm) | 86 | 76–94 | 86 | 79–99 | 81 | 73–88 | 0·251 | (1) $v.$ (2) | 0·517 |
| | | | | | | | | (1) $v.$ (3) | 0·009 |
| | | | | | | | | (2) $v.$ (3) | <0·002 |
| Hip circumference (cm) | 106·0 | 97–113 | 106·5 | 98–116 | 96·5 | 90–106 | <0·001 | (1) $v.$ (2) | 0·497 |
| | | | | | | | | (1) $v.$ (3) | <0·001 |
| | | | | | | | | (2) $v.$ (3) | <0·001 |

ARV, antiretroviral; IQR, interquartile range; SES, socio-economic status; ND, not determined; NA, not applicable.
Data are presented as median and interquartile range unless otherwise indicated.
*Multiple regression analysis adjusted for age, $P < 0·05$ indicates significance.
†Kruskal–Wallis test, $P < 0·05$ indicates significance.
‡$\chi^2$ test, $P < 0·05$ indicates significance.

**Table 2** Body composition variables according to study group: premenopausal black women, Soweto, Johannesburg, South Africa

| | HIV⁻ (*n* 97) (1) | | HIV⁺ non-ARV (*n* 74) (2) | | HIV⁺ pre-ARV (*n* 74) (3) | | | | |
|---|---|---|---|---|---|---|---|---|---|
| DXA (*n* 245) | Median | IQR | Median | IQR | Median | IQR | *P**, HIV⁻ *v.* HIV⁺ | Between-group comparison | *P** |
| WBLH fat mass (kg) | 24·45 | 17·35–30·57 | 25·31 | 18·06–32·88 | 18·43 | 12·45–25·32 | 0·002 | (1) *v.* (2) | 0·458 |
| | | | | | | | | (1) *v.* (3) | <0·001 |
| | | | | | | | | (2) *v.* (3) | <0·001 |
| WBLH lean mass (kg) | 37·68 | 34·21–41·28 | 39·67 | 34·86–42·26 | 36·18 | 33·56–39·84 | 0·201 | (1) *v.* (2) | 0·512 |
| | | | | | | | | (1) *v.* (3) | 0·005 |
| | | | | | | | | (2) *v.* (3) | 0·001 |
| Percentage body fat | 39·55 | 34·07–43·22 | 39·50 | 33·06–44·67 | 32·24 | 26·79–41·21 | <0·001 | (1) *v.* (2) | 0·372 |
| | | | | | | | | (1) *v.* (3) | <0·001 |
| | | | | | | | | (2) *v.* (3) | <0·001 |
| Fat mass:lean mass² (kg/kg²) | 17·19 | 13·76–20·38 | 15·92 | 13·36–19·85 | 13·84 | 10·15–18·88 | <0·003 | (1) *v.* (2) | 0·181 |
| | | | | | | | | (1) *v.* (3) | <0·001 |
| | | | | | | | | (2) *v.* (3) | 0·021† |
| Trunk fat mass (kg) | 9·72 | 6·68–13·56 | 11·38 | 7·39–15·28 | 7·48 | 4·64–11·81 | 0·007 | (1) *v.* (2) | 0·592 |
| | | | | | | | | (1) *v.* (3) | <0·001 |
| | | | | | | | | (2) *v.* (3) | <0·001 |
| Percentage trunk fat | 41·56 | 37·67–45·65 | 42·85 | 38·54–46·38 | 40·92 | 35·75–47·07 | 0·717 | (1) *v.* (2) | 0·602 |
| | | | | | | | | (1) *v.* (3) | 0·259 |
| | | | | | | | | (2) *v.* (3) | 0·117 |
| Limb fat mass (kg) | 14·00 | 10·32–16·70 | 13·97 | 10·74–17·93 | 10·75 | 7·32–14·15 | <0·001 | (1) *v.* (2) | 0·375 |
| | | | | | | | | (1) *v.* (3) | <0·001 |
| | | | | | | | | (2) *v.* (3) | <0·001 |
| Percentage limb fat | 58·44 | 54·35–62·33 | 57·15 | 53·62–61·46 | 59·08 | 52·93–64·25 | 0·717 | (1) *v.* (2) | 0·602 |
| | | | | | | | | (1) *v.* (3) | 0·259 |
| | | | | | | | | (2) *v.* (3) | 0·117 |
| Trunk:limb fat mass | 0·71 | 0·60–0·84 | 0·75 | 0·63–0·86 | 0·69 | 0·56–0·89 | 0·907 | (1) *v.* (2) | 0·567 |
| | | | | | | | | (1) *v.* (3) | 0·445 |
| | | | | | | | | (2) *v.* (3) | 0·204 |

DXA, dual energy X-ray absorptiometry; ARV, antiretroviral; IQR, interquartile range, WBLH, whole body less head.
*Multiple regression analysis adjusted for age, $P<0·05$ indicates significance.
†Non-significant due to Bonferroni adjustment.





**Table 3** Multiple regression analyses for BMI, WBLH fat mass, WBLH lean mass, trunk fat mass and limb fat mass: premenopausal black women, Soweto, Johannesburg, South Africa

| | *β* | 95% CI | *P* | | |
|---|---|---|---|---|---|
| BMI (kg/m²) | | | | | |
| Age | 0·257 | 0·146, 0·367 | <0·001 | | |
| HIV⁻ (ref.) | 0 | – | | $R^2$ | 0·14 |
| HIV⁺ non-ARV | −0·691 | −2·610, 1·227 | 0·479 | Adjusted $R^2$ | 0·13 |
| HIV⁺ pre-ARV | −4·487 | −6·394, −2·575 | <0·001 | *P* | <0·001 |
| WBLH fat mass (kg) | | | | | |
| Age | 0·347 | 0·166, 0·527 | <0·001 | | |
| HIV⁻ (ref.) | 0 | – | | $R^2$ | 0·137 |
| HIV⁺ non-ARV | −1·614 | −4·767, 1·539 | 0·314 | Adjusted $R^2$ | 0·123 |
| HIV⁺ pre-ARV | −7·884 | −11·014, −4·753 | <0·001 | *P* | <0·001 |
| WBLH lean mass (kg) | | | | | |
| Age | 0·214 | 0·128, 0·300 | <0·001 | | |
| Height | 50·826 | 40·207, 61·445 | <0·001 | | |
| HIV⁻ (ref.) | 0 | – | | $R^2$ | 0·342 |
| HIV⁺ non-ARV | −0·439 | −1·949, 1·070 | 0·567 | Adjusted $R^2$ | 0·331 |
| HIV⁺ pre-ARV | −3·411 | −4·910, −1·912 | <0·001 | *P* | <0·001 |
| Trunk fat mass (kg) | | | | | |
| Age | 0·208 | 0·116, 0·300 | <0·001 | | |
| HIV⁻ (ref.) | 0 | – | | $R^2$ | 0·134 |
| HIV⁺ non-ARV | −0·621 | −2·232, 0·990 | 0·448 | Adjusted $R^2$ | 0·12 |
| HIV⁺ pre-ARV | −3·521 | −5·121, −1·921 | <0·001 | *P* | <0·001 |
| Limb fat mass (kg) | | | | | |
| Age | 0·139 | 0·043, 0·234 | 0·004 | | |
| Height | 12·128 | 0·417, 23·84 | 0·042 | | |
| HIV⁻ (ref.) | 0 | – | | $R^2$ | 0·129 |
| HIV⁺ non-ARV | 0·993 | −2·658, 0·672 | 0·241 | Adjusted $R^2$ | 0·114 |
| HIV⁺ pre-ARV | −4·362 | −6·016, −2·710 | <0·001 | *P* | <0·001 |

WBLH, whole body less head; ref., reference category; ARV, antiretroviral.
All variables included in the above models were those which showed significance in prior analyses, $P<0·05$.

battered fried chicken). In addition, fish did not feature in the top twenty food items. Use of fats and oils was very common, with brick/hard margarine and sunflower oil being the regularly reported products (13 g/d and 6 g/d, respectively). Condiments, high in fat and/or sugar, were regularly added to meals and snacks, with both *atchar* (a spicy condiment of mangoes and honey, 19 g/d) and tomato sauce (10 g/d) featuring in the top twenty food items consumed.

## Discussion

In this sample of black, urban, SA women, HIV-positive individuals had lower weight and BMI, as well as lower fat and lean masses and percentage body fat, than their HIV-negative counterparts. This was primarily as a result of the HIV-positive individuals with low CD4 counts having low measures. Multiple regression analyses showed that HIV⁺ pre-ARV status, but not HIV⁺ non-ARV status, was a key contributor to differences in WBLH fat mass and WBLH lean mass, as well as both trunk and limb fat masses; associated with 8 kg and 3 kg less fat and lean mass, respectively, as well as 3·5 kg less trunk fat and 4 kg less limb fat. This challenges the stereotypical view of HIV as a disease associated with involuntary weight loss and wasting prior to ARV initiation and suggests that weight loss may only become a symptom in this population at more severe disease states.

Although WBLH fat mass, as well as trunk and limb fat masses, were lower in the HIV⁺ pre-ARV group than the other study groups, there were no differences in relative terms (trunk and limb fat percentages) across all groups. This, together with the similarity found in trunk:limb fat mass between study groups, suggests lower body fat across all sites rather than an altered fat distribution with advanced HIV infection. This contradicts previous US data which showed an increase in both percentage trunk fat and trunk:limb fat and a decrease in peripheral fat independent of ARV treatment in HIV-positive women compared with HIV-negative controls[13]. In addition, it suggests a different pattern of fat loss than that associated with ARV treatment, where lipodystrophy is characterised by abdominal fat accumulation and subcutaneous fat loss, predominantly at the face, limbs and buttocks[30]. This is speculative, however, and a longitudinal study is currently being undertaken in this population which it is hoped will provide more definitive answers.

As previously documented by Hamill *et al.*[20], the HIV⁺ pre-ARV group had a lower fat mass:lean mass² than the other two study groups, demonstrating approximately 21% and 16% less fat for each kilogram of lean mass than the HIV⁻ and HIV⁺ non-ARV groups, respectively. This provides evidence of lower fat mass, rather than lean tissue, at more advanced stages of HIV infection. Although previous literature has found HIV to be associated with preferential loss of lean compared

**Table 4** Daily energy and macronutrient intakes according to study group and comparison with dietary recommendations: premenopausal black women, Soweto, Johannesburg, South Africa

| | HIV⁻ (*n* 98) | | HIV⁺ non-ARV (*n* 74) | | | HIV⁺ pre-ARV (*n* 75) (3) | | | |
|---|---|---|---|---|---|---|---|---|---|
| Variable | Median | IQR | Median | IQR | *P**, HIV⁻ *v.* HIV⁺ | *P*†, HIV⁻ *v.* HIV⁺ | Median | IQR | Dietary Reference Intake[28] |
| Energy (kJ/d) | 11 863 | 10 179–14 404 | 12 225 | 10 541–14 767 | 0·121 | – | 11 773 | 9911–16 066 | 10 093‡ |
| Total digestible carbohydrate (g/d) | 392 | 329–459 | 404 | 339–503 | 0·210 | 0·825 | 388 | 331–487 | 130§ |
| Total fibre (g/d) | 27 | 20–34 | 27 | 20–36 | 0·363 | 0·916 | 26 | 20–37 | 25‖ |
| Total sugars (g/d) | 80 | 56–109 | 86 | 56–109 | 0·132 | 0·310 | 82 | 59–120 | |
| Total fat (g/d) | 97 | 83–124 | 100 | 78–130 | 0·331 | 0·447 | 88 | 71–140 | |
| Saturated fat (g/d) | 27 | 22–33 | 29 | 23–36 | 0·103 | 0·519 | 24 | 18–43 | |
| Polyunsaturated fat (g/d) | 31 | 25–40 | 27 | 21–39 | 0·877 | 0·09 | 28 | 22–43 | |
| Total protein (g/d) | 81 | 65–118 | 84 | 71–110 | 0·023 | 0·078 | 79 | 64–96 | 46§ |
| Plant protein (g/d) | 39 | 31–48 | 38 | 30–48 | 0·559 | 0·339 | 40 | 32–55 | |
| Animal protein (g/d) | 39 | 24–48 | 40 | 32–54 | 0·015 | 0·061 | 36 | 23–63 | |

ARV, antiretroviral; IQR, interquartile range.
*Multiple regression analysis adjusted for age. $P < 0{\cdot}05$ indicates significance.
†Multiple regression analysis adjusted for age and total energy intake (kJ). $P < 0{\cdot}05$ indicates significance.
‡Estimated Energy Requirement for adult woman aged 19–50 years.
§RDA for adult woman aged 19–50 years.
‖Adequate Intake for adult woman aged 19–50 years.

with fat tissue, particularly in male subjects, a disproportionately higher loss of fat mass has been shown in US women[31]. In addition, a preferential loss of fat rather than lean tissue mass has been demonstrated in males with high body fat percentages at baseline, compared with those having less than 15 % body fat[32].

Overweight and obesity were common in the sample overall, with a combined prevalence of 59 %, a prevalence higher than the national estimate of 55 % reported for black women in the 2003 SADHS[4]. Although the distribution of women across BMI cut-offs differed significantly between the HIV⁺ pre-ARV group and the other two study groups, there was still a 44 % prevalence of overweight and obesity in this group, while another 11 % were classified as underweight. Even at more advanced stages of HIV infection, women are affected by obesity and this needs to be addressed in the population as a whole.

There were no reported differences in dietary intake across study groups, with the exception of total and animal protein intakes which were lower in the HIV⁻ group than in the HIV⁺ groups combined. However, when adjusted for age and total energy intake, these differences were no longer significant. There was no relationship between protein intake and SES ($P = 0{\cdot}202$). These results also highlight that all groups, including the pre-ARV therapy group with lower median BMI, consume an obesogenic diet. Given the high levels of inflammation and high carbohydrate intake, these participants are likely to have a high prevalence of insulin resistance and other metabolic abnormalities. These are areas for potential future research.

Food item and food group analyses showed similar consistency of consumption across the groups, regardless of HIV status. The main contributor to the variation in food mass consumed between the three groups seemed to be 'pap' (maize meal porridge), which differed by approximately 135 g/d between the HIV⁺ pre-ARV group and the HIV⁻ group and approximately 92 g/d between the HIV⁺ pre-ARV group and the HIV⁺ non-ARV group. Diets in the sample as a whole were very high in refined carbohydrate, which is reflected by the high total digestible carbohydrate intake (approximately three-fold higher than the RDA of 130 g/d). Consumption of processed and fast-food products was common and that of fruits and vegetables rare, with the vegetable items consumed (e.g. tomato and onion stew) usually containing added sugar and/or fat. In addition to added sugar intakes, high sugar-based products were also common; with carbonated soft drinks featuring second in the top twenty food items consumed. This is a concern due to the link seen between sugar-sweetened beverage intake and weight gain, as well as diabetes and CVD risk[33,34]. The lack of fish, and therefore *n*-3 PUFA, in the top twenty reported food items should also be addressed due to the important role that these essential fatty acids have in regulating immune function[35]. The highly processed nature of food products, as well as high sugar and fat and



**Table 5** Top twenty food items consumed in the sample of premenopausal black women, Soweto, Johannesburg, South Africa

| | Food item | Food group* | Mean intake (g/d) |
|---|---|---|---|
| 1. | Maize meal porridge (cooked stiff/'pap') | Cereal and cereal products | 258 |
| 2. | Carbonated cold drink (e.g. Coca Cola) | Sugar, syrups and sweets | 197 |
| 3. | Brown bread/rolls | Cereal and cereal products | 121 |
| 4. | Full fat milk | Milk and milk products | 84 |
| 5. | White bread/rolls | Cereal and cereal products | 69 |
| 6. | White rice | Cereal and cereal products | 72 |
| 7. | Banana | Fruit | 53 |
| 8. | Apple | Fruit | 46 |
| 9. | French fries | Vegetables | 34 |
| 10. | Tomato and onion (stewed) | Vegetables | 22 |
| 11. | Chicken, dark meat (fried/roasted) | Meat and meat products | 20 |
| 12. | *Atchar* (mango and honey) | Sauces, seasonings and flavourings | 19 |
| 13. | Granulated white sugar | Sugar, syrups and sweets | 15 |
| 14. | Brick/hard margarine | Fats and oils | 13 |
| 15. | Tomato sauce | Sauces, seasonings and flavourings | 10 |
| 16. | Batter dipped fried chicken (e.g. KFC) | Meat and meat products | 9 |
| 17. | Cheddar cheese | Milk and milk products | 8 |
| 18. | Polony | Meat and meat products | 7 |
| 19. | Sweets (hard boiled/soft jelly type) | Sugar, syrups and sweets | 6 |
| 20. | Sunflower oil | Fats and oils | 6 |

*Food items grouped according to the current South African Medical Research Council food composition tables[37].

low vegetable consumption in the sample is an important health issue and highlights the urgent need to address obesity and its risk factors throughout the population.

Although providing an overall picture of habitual energy and macronutrient intakes, as well as of commonly consumed food items, the dietary data had certain limitations. The proportion of women classified as 'under-reporters' was very high (31 %, data not shown), suggesting that a substantial number were underestimating their consumption. This may have been due to the high prevalence of overweight and obesity in the sample, as high BMI has previously been shown to predict under-reporting[36]. Due to the negative impact that excluding these participants would have had on sample size, truncation was therefore chosen as the best possible method of minimising the effects of under-reporting on study results. Classification of food items based on the SA food composition table was also flawed as it misclassified items such as French fries into the vegetable group, thereby overestimating vegetable consumption in the sample. This classification should be revised in the future to ensure that vegetables high in starch with added fat are more accurately categorised.

A key limitation of the study was that the participants were not a random sample of the adult female population of Soweto and the surrounding area, as inclusion in the study required the women to present at the Chris Hani Baragwanath Hospital's Perinatal HIV Research Unit, HIV clinic, or voluntary counselling and testing centre. Women must, therefore, both know that the services exist and have access to them, must be healthy enough to present at the hospital, and must have sufficient information on HIV and its risks to be motivated to seek counselling or care. This could mean that the HIV-positive and HIV-negative participants included in the study are not a truly representative sample and may also have better knowledge of poor dietary practices and other health risks than the wider population.

Regardless of these weaknesses, the present paper provides unique data on diet and body composition differences between HIV-negative and HIV-positive women at varying levels of immunosuppression prior to treatment. In addition, the longitudinal design of the broader study will allow for future analysis of the changes in anthropometric, body composition and dietary variables in this population at 6 months, 12 months and 24 months follow-up.

## Conclusion

Our data show that immunosuppression may be a predictor of anthropometric and body composition changes in HIV-positive women and that, in populations with high obesity prevalence, these differences become evident only at advanced stages of infection. This highlights the need for a change in the way diet and body composition are viewed in HIV-positive patients, while emphasising that poor dietary habits should be addressed in the SA female, urban population as a whole.

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interest. *Authors' contributions:* The study was designed by M.M.H. and A.P. and the study design was reviewed by M.M.H., J.M.P., S.A.N. and A.P. S.V.W. and A.B.F. analysed the data. The initial phase of data analysis was conducted as part of S.V.W.'s MSc research project at the London School of Hygiene and Tropical Medicine. All authors contributed to interpretation and the writing of the manuscript. All authors had full access to the data. S.A.N. had responsibility for the final decision to submit the manuscript for publication. *Acknowledgements:* The authors wish to acknowledge all of the study participants and staff at the Developmental Pathways for Health Research Unit, ZAZI/Perinatal HIV Research Unit, Nthabiseng and Lilian Ngoyi clinics, Johannesburg, South Africa.

## References

1. Crum-Cianflone N, Roediger MP, Eberly L *et al.* (2010) Increasing rates of obesity among HIV-infected persons during the HIV epidemic. *PLoS One* **5**, e10106.
2. Tate T, Willig AL, Willig JH *et al.* (2012) HIV infection and obesity: where did all the wasting go? *Antivir Ther* **17**, 1281–1289.
3. Joint United Nations Programme on HIV/AIDS (2010) *UNAIDS Report on the Global AIDS Epidemic 2009.* Geneva: UNAIDS.
4. Department of Health, Medical Research Council & ORC Macro (2007) *South Africa Demographic and Health Survey 2003.* Pretoria: Department of Health.
5. Crum-Cianflone N, Tejidor R, Medina S *et al.* (2008) Obesity among patients with HIV: the latest epidemic. *AIDS Patient Care STDS* **22**, 925–930.
6. Ferrando SJ, Rabkin JG, Lin SH *et al.* (2005) Increase in body cell mass and decrease in wasting are associated with increasing potency of antiretroviral therapy for HIV infection. *AIDS Patient Care STDS* **19**, 216–223.
7. Mallon PW, Miller J, Cooper DA *et al.* (2003) Prospective evaluation of the effects of antiretroviral therapy on body composition in HIV-1-infected men starting therapy. *AIDS* **17**, 971–979.
8. Mwamburi DM, Wilson IB, Jacobson DL *et al.* (2005) Understanding the role of HIV load in determining weight change in the era of highly active antiretroviral therapy. *Clin Infect Dis* **40**, 167–173.
9. Dave JA, Lambert EV, Badri M *et al.* (2011) Effect of nonnucleoside reverse transcriptase inhibitor-based antiretroviral therapy on dysglycemia and insulin sensitivity in South African HIV-infected patients. *J Acquir Immune Defic Syndr* **57**, 284–289.
10. Esposito F, Coutsoudis A, Visser J *et al.* (2008) Changes in body composition and other anthropometric measures of female subjects on highly active antiretroviral therapy (HAART): a pilot study in KwaZulu-Natal, South Africa. *Southern Afr J HIV Med* **9**, 36–42.
11. Mercier S, Gueye NF, Cournil A *et al.* (2009) Lipodystrophy and metabolic disorders in HIV-1-infected adults on 4- to 9-year antiretroviral therapy in Senegal: a case–control study. *J Acquir Immune Defic Syndr* **51**, 224–230.
12. Despres JP (2007) Cardiovascular disease under the influence of excess visceral fat. *Crit Pathw Cardiol* **6**, 51–59.
13. Hadigan C, Miller K, Corcoran C *et al.* (1999) Fasting hyperinsulinemia and changes in regional body composition in human immunodeficiency virus-infected women. *J Clin Endocrinol Metab* **84**, 1932–1937.
14. George JA, Venter WD, Van Deventer HE *et al.* (2009) A longitudinal study of the changes in body fat and metabolic parameters in a South African population of HIV-positive patients receiving an antiretroviral therapeutic regimen containing stavudine. *AIDS Res Hum Retroviruses* **25**, 771–781.
15. Macallan DC, Noble C, Baldwin C *et al.* (1995) Energy expenditure and wasting in human immunodeficiency virus infection. *N Engl J Med* **333**, 83–88.
16. Hattingh Z, Walsh CM, Veldman FJ *et al.* (2006) Macronutrient intake of HIV-seropositive women in Mangaung, South Africa. *Nutr Res* **26**, 53–58.
17. Arendt BM, Aghdassi E, Mohammed SS *et al.* (2008) Dietary intake and physical activity in a Canadian population sample of male patients with HIV infection and metabolic abnormalities. *Curr HIV Res* **6**, 82–90.
18. Duran AC, Almeida LB, Segurado AA *et al.* (2008) Diet quality of persons living with HIV/AIDS on highly active antiretroviral therapy. *J Hum Nutr Diet* **21**, 346–350.
19. Hendricks KM, Willis K, Houser R *et al.* (2006) Obesity in HIV-infection: dietary correlates. *J Am Coll Nutr* **25**, 321–331.
20. Hamill M, Ward K, Pettifor J *et al.* (2013) Bone mass, body composition and vitamin D status of ARV-naïve, urban, black South African women with HIV infection, stratified by CD4 count. *Osteoporos Int* (Epublication ahead of print version).
21. Nel J & Steyn JP (2002) *Report on South African Food Consumption Studies Undertaken among Different Population Groups (1983–2000): Average Intakes of Foods Most Commonly Consumed.* Pretoria: Department of Health.
22. Zingoni C, Norris SA, Griffiths PL *et al.* (2009) Studying a population undergoing nutrition transition: a practical case study of dietary assessment in urban South African adolescents. *Ecol Food Nutr* **48**, 178–198.
23. Steyn N & Senekal M (2005) *A Guide for the Use of the Dietary Assessment and Education Kit (DAEK).* Cape Town: Medical Research Council.
24. Steyn NP, Senekal M, Norris SA *et al.* (2006) How well do adolescents determine portion sizes of foods and beverages? *Asia Pac J Clin Nutr* **15**, 35–42.
25. Langenhoven ML, Kruger M, Gouws E *et al.* (1991) *MRC Food Composition Tables*, 3rd ed. Cape Town: Medical Research Council.
26. Goldberg GR, Black AE, Jebb SA *et al.* (1991) Critical evaluation of energy intake data using fundamental principles of energy physiology: 1. Derivation of cut-off limits to identify under-recording. *Eur J Clin Nutr* **45**, 569–581.
27. Black AE (2000) Critical evaluation of energy intake using the Goldberg cut-off for energy intake:basal metabolic rate. A practical guide to its calculation, use and limitations. *Int J Obes Relat Metab Disord* **24**, 1119–1130.
28. Food and Nutrition Board, Institute of Medicine (2002) *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids.* Washington, DC: The National Academies Press.
29. McVeigh JA, Norris SA & de Wet T (2004) The relationship between socio-economic status and physical activity patterns in South African children. *Acta Paediatr* **93**, 982–988.
30. James J, Carruthers A & Carruthers J (2002) HIV-associated facial lipoatrophy. *Dermatol Surg* **28**, 979–986.
31. Grinspoon S, Corcoran C, Miller K *et al.* (1997) Body composition and endocrine function in women with acquired immunodeficiency syndrome wasting. *J Clin Endocrinol Metab* **82**, 1332–1337.
32. Mulligan K, Tai VW & Schambelan M (1997) Cross-sectional and longitudinal evaluation of body composition in men with HIV infection. *J Acquir Immune Defic Syndr Hum Retrovirol* **15**, 43–48.
33. Hu FB & Malik VS (2010) Sugar-sweetened beverages and risk of obesity and type 2 diabetes: epidemiologic evidence. *Physiol Behav* **100**, 47–54.
34. Malik VS, Popkin BM, Bray GA *et al.* (2010) Sugar-sweetened beverages, obesity, type 2 diabetes mellitus, and cardiovascular disease risk. *Circulation* **121**, 1356–1364.
35. Calder PC & Yaqoob P (2009) Omega-3 polyunsaturated fatty acids and human health outcomes. *Biofactors* **35**, 266–272.
36. Price GM, Paul AA, Cole TJ *et al.* (1997) Characteristics of the low-energy reporters in a longitudinal national dietary survey. *Br J Nutr* **77**, 833–851.
37. Wolmarans P, Danster N, Dalton A *et al.* (editors) (2010) *Condensed Food Composition Tables for South Africa.* Cape Town: Medical Research Council.

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ORIGINAL ARTICLE

# Bone mass, body composition and vitamin D status of ARV-naïve, urban, black South African women with HIV infection, stratified by $CD_4$ count

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**Abstract**
*Summary* This is the first report examining vitamin D status and bone mass in African women with HIV infection using dual-energy X-ray absorptiometry (DXA) with an appropriate HIV-negative control group. Unlike previous publications, it demonstrates no difference in bone mineral density (BMD) or vitamin D status in HIV-positive patients, at different disease stages, vs. HIV-negative subjects.
*Introduction* Low bone mass and poor vitamin D status have been reported among HIV-positive patients; suggesting HIV or its treatment may increase the risk of osteoporosis, a particular concern for women in countries with high HIV prevalence such as South Africa. We describe bone mass and vitamin D status in urban premenopausal South African women, who were HIV positive but not on antiretroviral therapy (ARV).
*Methods* This study is a cross-sectional measurement of BMD and body composition by DXA and vitamin D status by serum 25-hydroxyvitamin D (25(OH)D) concentration. Subjects were recruited into three groups: HIV negative ($n$=98) and HIV positive with preserved $CD_4$ cell count (non-ARV; $n$=74) or low $CD_4$ cell counts prior to ARV initiation (pre-ARV; $n$=75).
*Results* The mean (standard deviation (SD)) age of women was 32.1 (7.2) years. Mean $CD_4$ (SD) counts ($\times 10^6$/l) were 412 (91) and 161 (69) in non-ARV and pre-ARV groups ($p<0.0001$). Pre-ARV women were significantly lighter and had lower mean BMI than the other two groups ($p<0.002$). The pre-ARV group also had significantly less fat and lean mass compared with non-ARV and HIV-negative subjects ($p\leq 0.05$). After full adjustment, there were no significant differences in BMD at any site ($p>0.05$) between the groups, nor was vitamin D status significantly different between groups ($p>0.05$); the mean (SD) cohort 25(OH)D being 60 (18) nmol/l.
*Conclusion* Contrary to previous studies, these HIV-positive women did not have lower BMD or 25(OH)D concentrations than HIV-negative controls, despite the pre-ARV group being lighter with lower BMI.

**Keywords** Body composition · Bone mineral density · Dual energy X-ray absorptiometry · HIV infection · Vitamin D

## Introduction

HIV infection and the use of antiretroviral (ARV) medication have been associated with low bone mineral density (BMD) and poor vitamin D status. In a meta-analysis, the prevalence of low BMD in HIV-positive individuals was three times higher than in HIV-negative controls [1–3]. Similarly, studies have described high prevalence of low 25-hydroxyvitamin D

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(25(OH)D) concentrations in HIV-positive patients [4]. Some studies of the effects of HIV and/or its treatment on bone are limited by retrospective design, a preponderance of white, male subjects, and lack of HIV-negative controls [5] while others are prospective [6] and do include women [7, 8]. Other studies are limited by confounding by low body weight or other risk factors for low BMD, such as intravenous drug use (IDU), exposure to a large variety of ARV regimes and measurement of BMD and vitamin D status after varying duration of ARV exposure [6]. The few prospective studies focusing on women have also been limited by some of these aspects [6, 9], and as a result it is difficult to ascertain with certainty if HIV infection and/or its treatment or factors unrelated to HIV infection are contributing factors to the low bone mass and low vitamin D status described in the current literature. In contrast, there are data to suggest that after adjusting for body weight, BMD is normal or near normal, and that patients on ARV do not have increased rates of bone loss [10, 11]. As a result, there is not a definitive consensus on the contribution of HIV infection or ARV exposure on BMD in infected individuals.

In South Africa, estimates of HIV prevalence for 2010 are 10.5 % for the total population and 29.3 % for women attending antenatal clinics. The epidemic is described as "hyperendemic" because of the high prevalence and continuing drivers of transmission [12–14]. In South Africa, individuals generally become eligible for ARV treatment when their $CD_4$ count is less than a nationally specified threshold. By 2009, 56 % of those requiring ARV were able to receive them, with the government intending to increase ARV coverage to 80 % by 2011 [12].

Vitamin D has well-known associations with bone health via its role in calcium and phosphate homeostasis, and vitamin D status is considered an important modulator of immune function by some authors [14–16]. In South Africa, adults are largely dependent on the cutaneous synthesis of vitamin D to maintain vitamin D status, as only small amounts of vitamin D are obtained from the diet due to limited food fortification. In Johannesburg (26° S latitude), there is sufficient ultraviolet B (UVB) radiation in sunshine throughout the year for dermal synthesis of vitamin D [17]. Nevertheless, vitamin D deficiency has been described in tropical/subtropical countries despite the potential for adequate skin exposure to UVB-containing sunshine [18].

The aim of the study presented here was to describe BMD, body composition and vitamin D status in South African women with and without HIV infection, prior to a planned longitudinal study of this cohort to chart the changes in these outcomes over time. We hypothesised that HIV-positive women with low $CD_4$ counts, below the threshold that would make them eligible for ARV treatment, would have lower bone mass, less fat and muscle mass and inferior vitamin D status than HIV-positive women with preserved $CD_4$ counts and HIV-negative women in South Africa.

## Methods

### Subjects

Urban, black, premenopausal, South African women ($n$=247) were recruited from clinics in Soweto, Greater Johannesburg and enrolled into the study between February and July 2010. Subjects were recruited from a voluntary counselling and testing clinic and local health clinics. The aim was to recruit 95 HIV-negative and 73 (±10) in each of two HIV-positive groups (with or without low $CD_4$ counts). This sample size was based on calculations for the longitudinal study to detect a 2 % change in lumbar spine BMD, allowing for a between-individual coefficient of variation in BMD of 5 %, with 95 % confidence and 80 % power. For the study presented here, this sample size was sufficient for a comparison of three groups to allow the detection of mean differences between each pair of groups of around 0.4 standard deviation (SD) at 5 % significance and 80 % power. The study was approved by the University of the Witwatersrand Human Research Ethics Committee (HREC number: M101525) and the Gauteng Department of Health.

Eligible subjects were adult females (defined as aged greater than 18 years) and premenopausal (defined as regular menses). Other inclusion criteria included a documented negative HIV test within the last 12 weeks for HIV-negative women and a documented positive HIV test for all other women. Patient-retained clinic records were scrutinised whenever possible to confirm medical history, current $CD_4$ count, prior exposure to ARVs and concurrent medication use. Exclusion criteria included conditions associated with abnormal bone metabolism or current use of medication likely to affect bone or vitamin D status such as bisphosphonates. Pregnant and lactating women were excluded as were those with an acute medical condition. The group with the lowest $CD_4$ count were largely recruited after the other groups: May to June and February to April, respectively.

Study posters were displayed in the clinic and training sessions undertaken with clinic staff. Women who expressed an interest in the study underwent initial telephone screening, in their language, to ensure inclusion and exclusion criteria were met. Prior to enrolment, potential subjects completed a medical- and health-related questionnaire to assess past and current health status and medication use and to further assess compliance with inclusion and exclusion criteria. Women who remained eligible were enrolled in the full study after they had provided written consent.

The enrolled women consisted of HIV-negative ($n$=98) and HIV-positive ($n$=149) subjects. The HIV-positive women were recruited into two prespecified groups: those with relatively preserved $CD_4$ counts ($>350\times10^6$ cells/l), not requiring ARV therapy (non-ARV group; $n$=74) and those with low $CD_4$ counts (in the region of $200\times10^6$ cells/l) requiring ARV initiation (pre-ARV group; $n$=75) according to the current South Africa (SA) treatment guidelines [19]. HIV-negative status was confirmed using the Determine™ rapid HIV-antibody test (Alere San Diego, Inc., San Diego, CA, USA), while HIV-positive status was established using a second platform. HIV-positive women were either newly diagnosed or known to be HIV positive, but not on ARVs. All HIV-positive women provided an up-to-date (within 3 months) $CD_4$ count prior to enrolling into the study. All HIV-positive women received SA standard of care with respect to ARV provision and clinical follow up. Women requiring urgent ARV initiation were managed in such a way that there would be no delay in ARV initiation if they were to participate in the study.

Women attended the Developmental Pathways for Health Research Unit (DPHRU) facility at the Chris Hani Baragwanath Academic Hospital, after an overnight fast and underwent phlebotomy, anthropometry, and dual-energy X-ray absorptiometry (DXA) assessment of bone mass and body composition. After phlebotomy, subjects were given breakfast and each received ZAR 50.00 (≈US$6.25) for travel expenses.

### Anthropometry

Height was measured to the nearest millimetre using a stadiometer (Holtain, Crosswell, UK). Weight was measured to the nearest 100 g using a digital scale (Tanita, TBF-410 MA Body Composition Analyzer, Tanita Corporation of America, Inc., Arlington Heights, IL, USA) with participants wearing light clothing and no shoes. BMI was calculated as the participant's weight in kilograms divided by the square of their height in metres (in kilogram per square metre). Underweight, normal, overweight, and obese were defined as BMI <18.5, 18.5–24.9, 25–29.9, ≥30.0 kg/m$^2$, respectively [20].

### Bone absorptiometry and body composition measurements

DXA was performed using a Hologic QDR 4500A DXA (model: Discovery W (S/N 71201) software version 12.5:7 Hologic, Inc., Waltham, MA, USA) according to standard procedures. Scans were conducted using the automatic scan mode, i.e. 'array', 'fast array' or 'slow array', depending on the weight of the subjects. Subjects wore light clothing having removed metal objects, jewellery, etc. DXA was used to measure bone mineral content (BMC, in grams), bone area (BA, in square centimetre) and areal BMD (in grams per square centimetre), of whole body (WB), total hip (TH), femoral neck (FN) and lumbar spine (LS). The coefficients of variation (CV%) for repeated measurements of the manufacturer's phantom were 0.3, 0.4 and 0.2 % for BA, BMC and BMD, respectively. The CV for repeated measurement by the DXA operator of the LS and TH BMD were 0.7 and 1.0 %, respectively. DXA scans for WB were analysed using WB less head (WBLH) as many women wore wigs and hair weaves that could not be removed prior to scanning. This artificial hair was of similar density to soft tissue and therefore could cause measurement artefact. Total fat and lean body mass (in grams) were also measured by DXA.

### Laboratory analysis

Blood was collected for 25(OH)D analysis, measured by chemi-luminescent immunoassay (Liason) kit (DiaSorin Inc., Stillwater, MN, USA). The blood samples were allowed to clot for a minimum of 20 min at room temperature, and the serum was aliquoted and stored at −20 C until analysed. All samples were run in duplicate. The inter-assay CV for low and higher 25(OH)D controls was 10 and 9 %, respectively, whereas the intra-assay CV was 8 and 6 %, respectively. The DPHRU laboratory participates in the International Vitamin D External Quality Assessment Scheme and holds the certificate of proficiency [21].

### Statistical analysis

Data were analysed using DataDesk 6.3.1 (Data Description Inc, Ithaca, NY, USA) and summary statistics were documented as mean (SD) or median (interquartile range), depending on the distribution. Comparisons were made between the three groups of women using hierarchical linear models; ANOVA (or ANCOVA) and Scheffé post hoc tests were used to compare group means (standard error (SE)). To consider the possible influence of group differences in bone and body size, bone mineral data were adjusted for age, weight, height and bone area, and bone area was adjusted for age, weight and height, using ANCOVA [16]. Preliminary plots of the relationship between fat mass and lean mass in this sample population demonstrated non-linearity. Regression of fat mass on lean mass in the HIV-negative control group with data in natural logarithms gave a power exponent 2.05± 0.18 (SE) , indicating that fat mass-to-lean square mass best described the relationship in this population. The exponent was similar when the data from all three groups were included in the model; 2.07±0.14. Consequently, a fat mass-to-lean square mass term was used to describe differences in body composition between the groups, and logarithmic regression was used to adjust fat mass for lean mass in statistical models. BMD SD scores (SDS) were generated using HIV-negative subjects as the reference population (ref) against which the SDS for each individual HIV-positive woman (i) was derived as follows: $[(BMD_i - \text{mean } BMD_{ref})/SD_{ref}]$. A $p$ value of ≤0.05 was considered to be statistically significant.

**Table 1** Subject characteristics, anthropometric measurements and vitamin D status as measured by serum 25(OH)D

| | Group 1 HIV-negative $n$=98 | Group 2 HIV-positive, non-ARV $n$=74 | Group 3 HIV-positive, pre-ARV $n$=75 | Group effect ANOVA $p$ |
|---|---|---|---|---|
| Age (years) | 30.0 (8.1) | 33.5 (6.1)[a] | 33.4 (6.5)[a] | 0.001 |
| HIV status | Negative | Positive | Positive | |
| Current $CD_4$ count $\times 10^6$ cells/l | ND | 412 (91) | 161 (69)[b] | <0.001 |
| Median (IQR) | | 420 (127;409) | 175 (120;165) | |
| Min | NA | 240 | 18 | |
| Max | NA | 604 | 275 | |
| Gravidity median (IQR) | 1 (0;2) | 2 (2;3)[a] | 2 (1;3)[a] | |
| Range | 0–5 | 0–6 | 0–6 | |
| Current hormonal contraceptive use (%) | 34 (35.4) | 26 (36.6) | 25 (33.3) | 0.9 |
| Current smoking (%) | 10.2 | 13.5 | 8 | 0.2 |
| Height (cm) | 157.6 (5.9) | 159.4 (5.9) | 159.2 (5.3) | 0.06 |
| Weight (kg) | 69.7 (17.0) | 72.0 (17.4) | 62.3 (15.2)[c,d] | <0.001 |
| BMI (kg/m$^2$) Median (IQR) | 27.3 (23.1;31.7) | 27.8 (23.3;32.3) | 23.5 (20.5;27.0)[d,e] | <0.001 |
| Overweight BMI >24.9 kg/m$^2$, <30 kg/m$^2$ (%) | 35 | 28 | 28 | |
| Obese BMI >30 kg/m$^2$ (%) | 30 | 37 | 16 | |
| Underweight BMI <18.5 kg/m$^2$ (%) | 4 | 1 | 11 | |
| WBLH Fat (kg) | 26.1 (11.5) | 26.1 (9.8) | 19.7 (9.3)[b,e] | <0.0001 |
| WBLH Lean (kg) | 38.3 (60.8) | 39.5 (62.4) | 36.4 (48.1)[d] | 0.005 |
| Fat/lean$^2$ (kg/kg$^2$)* | 17.32 (4.80) | 15.92 (4.56) | 14.58 (5.47)[a,f] | 0.002 |
| 25(OH)D (nmol/l) | 59.7 (16.5) | 59.2 (16.5) | 61.6 (22.3) | 0.7 |
| 25(OH)D (nmol/l) >50 (%) | 73.5 | 70.3 | 66.7 | |
| 25(OH)D (nmol/l) <50 (%) | 26.5 | 29.7 | 33.3 | |
| 25(OH)D (nmol/l) <25 (%) | 1.0 | 2.7 | 5.3 | |

All values are mean (SD) unless indicated. Letters are used to indicate significance of between-group differences as tested by ANOVA/Scheffé

*25(OH)D* 25 hydroxyvitamin D, *ARV* antiretroviral therapy, *cm* centimetres, *IQR* interquartile range, *kg* kilograms, *SD* standard deviation, *WBLH* whole body less head, *ND* not determined, *NA* not applicable

*Value multiplied by 1,000 to illustrate the relative differences in kilogram

[a] Significantly different from group 1, $p \leq 0.01$

[b] Significantly different from group 2, $p \leq 0.001$

[c] Significantly different from group 1, $p \leq 0.05$

[d] Significantly different from group 2, $p \leq 0.01$

[e] Significantly different from group 1, $p \leq 0.001$

[f] Significantly different from group 2, $p \leq 0.05$

## Results

### Subject characteristics

By design, the mean $CD_4$ count ($\times 10^6$ cells/l) in the pre-ARV group was significantly lower than that in the non-ARV group (412 (91) and 161 (69), respectively, $p<0.0001$). The mode of acquisition of HIV-infection was via heterosexual transmission in all subjects, only one subject reporting IDU in the past (Table 1).

Mean age (SD) was 32.1 (7.2) years with HIV-negative women being significantly but only slightly younger than both groups of HIV-positive women. The age ranges were similar in the three groups (18–49, 22–48 and 19–47 years in HIV-negative, non-ARV and pre-ARV women, respectively). Median (IQR) gravidity was 2 (1; 3) with both HIV-positive groups having a higher median gravidity compared to the HIV-negative group.

### Anthropometry and body composition

HIV-negative women tended to be shorter than both groups with HIV-infection ($p=0.06$), while HIV positive, pre-ARV women were significantly lighter than the other two groups ($p<0.05$). Median (IQR) BMI of the study cohort was 26.1 (22.4; 31) kg/m$^2$ with BMI in pre-ARV women being significantly lower than in HIV-negative and non-ARV women. Combined overweight and obesity represented 65, 65 and 44 % of subjects in HIV-negative, non-ARV and pre-ARV women, respectively , while underweight was present in 4, 1 and 11 %, respectively (Table 1).

There were significant differences in fat mass between groups with pre-ARV women having significantly lower fat mass than non-ARV women ($p \leq 0.001$). Although lean mass was also lower in pre-ARV compared with non-ARV women ($p=0.005$) the pre-ARV group had lower fat mass-to-lean square mass ratio than the other two groups ($p=0.002$). When fully adjusting for lean mass using logarithmic regression, the pre-ARV group had significantly lower fat mass for their lean mass than the other two groups; such that for each unit of lean mass the pre-ARV group had a mean difference (SE) of 21 (5)% less fat than the controls, $p=0.0002$, and 16 (5)% less fat than the non-ARV group, $p=0.02$.

### Bone measures

No significant differences in BMD at the TH, FN, LS and WBLH were found, and age and size adjustment did not reveal any differences between groups. When expressed as SD scores, there were no significant differences between pre-ARV and non-ARV groups in BMD for any site measured ($p>0.05$) and all the mean values were within a −0.5 SD of the HIV-negative reference group (Table 2). In addition, no significant differences were found in BMC values except at WBLH when fully adjusted for age, size and BA ($p=0.03$). Unadjusted BA was significantly greater in both groups of HIV-positive women than HIV-negative women at some sites but these differences disappeared after adjusting for age and size (see Electronic supplementary material (ESM) for BA and BMC data).

### Vitamin D status

Mean (SD) 25(OH)D for the whole cohort was 60.1 (18.4) nmol/l and there were no significant differences between groups ($p>0.05$). 25(OH)D concentration was <50 nmol/l in 29.6 % of individuals; with similar proportions in each of the groups in this category (26.5, 29.7 and 33.3 % in HIV-negative, non-ARV and pre-ARV, respectively). Very few subjects had a 25(OH)D concentration <25 nmol/l (1.0, 2.7 and 5.3 % in the three groups, respectively), despite the slightly greater number of pre-ARV subjects whose blood samples for 25(OH)D measurement were obtained during the winter months.

## Discussion

The aim of this study was to determine whether South African HIV-positive women with preserved $CD_4$ counts differed from those with low $CD_4$ counts making them eligible for ARV and to compare each group with HIV-negative women. In this group of urban, South African women, pre-ARV women were significantly lighter than HIV-negative and non-ARV subjects and had lower fat mass than expected for their lean mass, raising the possibility that women with advancing HIV disease preferentially lose fat rather than lean mass. There were no significant differences between groups in BMC or BMD at any site before or after adjustment for age, BA, weight and height and the observed smaller BA in the HIV-negative women disappeared after adjustment for age, height and weight. There was no significant difference in vitamin D status between groups with the majority of subjects having a serum concentration >50 nmol/l.

The assessment of 'optimal' vitamin D status is problematic because varying cut offs are used to define sufficiency, insufficiency and deficiency [22]. A concentration below 25 nmol/l is generally recognised as indicating an increased risk of rickets and osteomalacia [23]. The 2010 Institute of Medicine report considered that a blood 25(OH)D concentration of 20 ng/mL (50 nmol/l) to be sufficient for good bone health in 'practically all individuals' [24]. However, it noted that evidence was lacking to make a similar statement regarding non-skeletal health. In the context of HIV infection and ARV use, the optimal vitamin D status remains undefined because there

**Table 2** BMD of the three groups of South African women

| | BMD (g/cm$^2$) Mean (SD) | | | Group effect[a] $p$ |
|---|---|---|---|---|
| | Group 1 HIV-negative $n$=98 | Group 2 HIV-positive, non-ARV $n$=74 | Group 3 HIV-positive, pre-ARV $n$=75 | |
| Total Hip | 1.013 (0.131) | 0.985 (0.124) | 0.988 (0.125) | 0.3 |
| Femoral Neck | 0.930 (0.114) | 0.916 (0.125) | 0.923 (0.131) | 0.8 |
| Lumbar Spine | 1.018 (0.118) | 1.021 (0.109) | 1.006 (0.128) | 0.7 |
| WBLH | 0.958 (0.079) | 0.943 (0.071) | 0.947 (0.080) | 0.4 |

*ARV* antiretroviral therapy, *BMD* bone mineral density (in gram per square centimetre), *SD* standard deviation, *WBLH* whole body less head

[a] Group effect by ANOVA. There were no significant differences between pairs of groups by Scheffé post hoc tests

may be different requirements for maximal bone health and immune functioning compared with HIV-negative populations. However, in contrast to other reports [4, 25], in our study, there were no indications that HIV infection was associated with inferior vitamin D status because there were no significant differences in vitamin D status between the three groups, the distributions of 25(OH)D concentration were similar, and vitamin D status appeared to be generally adequate with very few women having a concentration <25 nmol/l.

Contrary to previous reports [9], we found no significant differences in BMD between either group of HIV-positive and HIV-negative women. Full adjustment for bone and body size did not alter these results. This lack of any differences is surprising as HIV-positive women with low $CD_4$ counts, requiring ARV initiation, were significantly lighter, with lower fat and lean mass, than the other women. However, it may reflect the selection criteria for this study because despite recruiting women with low $CD_4$ counts, of clinical concern, women with severe clinical disease received immediate ARV therapy and were thus excluded from the study. It may also be influenced by the fact that the subjects were not intravenous drug users and thus not exposed to the additional effect on BMD that this poses. Another limitation may be that the groups were different in terms of duration of hormonal contraception use, parity and total duration of lactation; however, at the time of the study, no women were pregnant or lactating. The findings are also limited by the fact that the sample of HIV-positive women was likely to be heterogeneous with respect to immune status and duration of infection. However, most other studies have also recruited HIV-positive subjects in a similar manner and this is unlikely to account for the different findings in our study.

The rates of combined overweight and obesity 65 % in HIV-negative and non-ARV subjects in this study were greater than the national average in South Africa of 51.5 % [26]; even women with advanced HIV-disease (pre-ARV group) had a combined overweight and obesity rate of 44 %. It is possible, therefore, that the typically high weight of South African women has a sparing effect on bone in those with HIV infection, even with $CD_4$ counts below the threshold for initiation of ARV intervention.

Historically, being overweight has been viewed as protective against osteoporotic fracture, although evidence is emerging that overweight and obesity may be a risk factor for leg fragility fractures in women [27]. In the study population of younger black women in South Africa, there were no significant differences in BMD SD score, expressed relative to the HIV-negative group, according to HIV status at any site. The effects of HIV and its treatment on fracture risk in South Africa are unknown.

The lack of difference between the groups which is at variance from previously reported studies may be the result of true lack of effect of HIV infection or reflect important differences in bone response to HIV between black Africans and Caucasians. The study design in which two distinct groups of HIV-positive women, based on South African eligibility criteria for ARV treatment plus the inclusion of a HIV-negative control group strengthens the finding that HIV infection with varying degree of immunosuppression does not appear to be driving alterations in BMD or vitamin D status in these young, urban women. The high rates of overweight may be masking more dramatic differences in BMD and vitamin D in those subjects with advanced clinical HIV disease not included in this study. Further work is required to address the effects of ARV exposure on bone and vitamin D status as well as the relative effect of 'traditional' osteoporosis risk factors in this population. The data from this study provide an insight into bone health, body composition and vitamin D status in African women living with HIV. They challenge our own hypotheses and previously reported differences in BMD and vitamin D status in HIV-positive subjects living in developed countries and highlight the importance of studying subjects prior to ARV exposure.

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**Conflicts of interest** There were no conflicts of interest.

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## References

1. Brown TT, McComsey GA (2006) Osteopenia and osteoporosis in patients with HIV: a review of current concepts. Curr Infect Dis Rep 8(2):162–170
2. Brown TT, Qaqish RB (2006) Antiretroviral therapy and the prevalence of osteopenia and osteoporosis: a meta-analytic review. AIDS 20(17):2165–2174
3. Brown TT et al (2004) Reduced bone mineral density in human immunodeficiency virus-infected patients and its association with increased central adiposity and postload hyperglycemia. J Clin Endocrinol Metab 89(3):1200–1206
4. Welz T et al (2010) Efavirenz is associated with severe vitamin D deficiency and increased alkaline phosphatase. AIDS 24(12):1923–1928
5. Bonjoch A et al (2010) High prevalence of and progression to low bone mineral density in HIV-infected patients: a longitudinal cohort study. AIDS 24(18):2827–2833
6. Dolan SE, Kanter JR, Grinspoon S (2006) Longitudinal analysis of bone density in human immunodeficiency virus-infected women. J Clin Endocrinol Metab 91(8):2938–2945
7. Yin M et al (2005) Bone mass and mineral metabolism in $HIV^+$ postmenopausal women. Osteoporos Int 16(11):1345–1352
8. Arnsten JH et al (2006) HIV infection and bone mineral density in middle-aged women. Clin Infect Dis 42(7):1014–1020
9. Dolan SE et al (2004) Reduced bone density in HIV-infected women. AIDS 18(3):475–483
10. Bolland MJ et al (2007) Low body weight mediates the relationship between HIV infection and low bone mineral density: a meta-analysis. J Clin Endocrinol Metab 92(12):4522–4528
11. Bolland MJ et al (2007) Bone mineral density remains stable in HAART-treated HIV-infected men over 2 years. Clin Endocrinol (Oxf) 67(2):270–275
12. Republic of South Africa. Country progress report on the declaration of commitment on HIV/AIDS 2010. Report – reporting period: January 2008 - December 2009. http://data.unaids.org/pub/report/2010/southafrica_2010_country_progress_report_en.pdf
13. Statistics South Africa (2010) Mid-year population estimates 2010: Pretoria South Africa. p. 1–16
14. Adams JS et al (2007) Vitamin D in defense of the human immune response. Ann N Y Acad Sci 1117:94–105
15. Conesa-Botella A et al (2009) Is vitamin D deficiency involved in the immune reconstitution inflammatory syndrome? AIDS Res Ther 6:4
16. Liu PT et al (2006) Toll-like receptor triggering of a vitamin D-mediated human antimicrobial response. Science 311(5768):1770–1773
17. Pettifor JM, Ross FP, Solomon L (1978) Seasonal variation in serum 25-hydroxycholecalciferol concentrations in elderly South African patients with fractures of femoral neck. Br Med J 1(6116):826–827
18. Schoenmakers I, Goldberg GR, Prentice A (2008) Abundant sunshine and vitamin D deficiency. Br J Nutr 99(6):1171–1173
19. National Department of Health South Africa (2010) Clinical guidelines for the management of HIV & AIDS in adults and adolescents. http://www.sahivsoc.org/upload/documents/Clinical_Guidelines_for_the_Management_of_HIV_AIDS_in_Adults_Adolescents_2010.pdf
20. WHO (2006) W.H.O. BMI classification
21. Poopedi MA, Norris SA, Pettifor JM (2011) Factors influencing the vitamin D status of 10-year-old urban South African children. Public Health Nutr 14(2):334–339
22. Prentice A, Goldberg GR, Schoenmakers I (2008) Vitamin D across the lifecycle:physiology and biomarkers. Am J Clin Nutr 88:500S–506S
23. Scientific Advisory Committee on Nutrition (2007) Update on vitamin D. Norwich: TSO (The Stationery Office)
24. Institute of Medicine (2010) Dietary reference intakes for calcium and vitamin D: National Academies Press
25. Van Den Bout-Van Den Beukel CJ et al (2008) Vitamin D deficiency among HIV type 1-infected individuals in the Netherlands: effects of antiretroviral therapy. AIDS Res Hum Retroviruses 24(11):1375–1382
26. Kruger HS et al (2011) Overweight among children decreased, but obesity prevalence remained high among women in South Africa, 1999–2005. Public Health Nutr 2012 Apr;15(4):594–9
27. Compston JE et al (2011) Obesity is not protective against fracture in postmenopausal women: GLOW. Am J Med 124(11):1043–1050



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